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Opportunities and Challenges for Sustainable Bioremediation of Natural and Synthetic Estrogens as Emerging Water Contaminants Using Bacteria, Fungi, and Algae

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Abstract The occurrence of newly emerging contaminants such as estrogens in water environment has the potential negative effects to human health as well as the surrounding wildlife. This demands efficient approaches for their removals from the water environment. Among all feasible solutions, biodegradation shows promising prospects to remediate estrogens from the environment since it is relatively economical and environmentally friendly compared to chemical and physical treatment approaches. To offer coverage on the present advances of this technology, this paper critically reviews the opportunities and challenges for bioremediation of estrogens using bacteria, fungi, and algae. In general, the capabilities to remove estrogens from water environments by bacteria, fungi, and algae have been highlighted and discussed. Additionally, several advantages and disadvantages are recognized before they are implemented widely in full-scale treatments. Moreover, a comprehensive discussion on the transformation of estrogens using these organisms is also presented, showing vividly that estrogens can be transformed into less toxic chemicals. The review ends by offering several prospective areas for expansion in the future specifically in focusing on the evaluation of other available microorganisms that can survive under numerous hostile environmental conditions, since, in the real application, complex mixtures and extreme environmental conditions are commonly observed particularly in the wastewater treatment systems.

Keywords Estrogens · Bioremediation · Biodegradation · Biotransformation

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1 Introduction

Pronounced quantity of estrogens in the aquatic environment presents a potential health hazard to water life, wildlife, as well as the general human

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population. Chronic intake from the source poses a direct internal sexual hormone balance issue to the consumers (Vymazal et al., 2015). In categorization, estrogens can be classified as natural and synthetic. In natural estrogens, they include estrone (E1), 17β-estradiol (E2), and estriol (E3). In addition, 17α-ethinylestradiol (EE2) and diethylstilbestrol (DES) are the category of synthetic estrogens. Natural estrogens are commonly found in vertebrates and insects (Chen et al., 2017b). In humans, estrogens are biologically produced in the adrenal cortex, testes, ovary, and placenta (Simpson, 2003). The synthetic pharmaceutical estrogens are commonly used for menopause estrogen replacement. Moreover, they are used in medical for birth control and abnormal sex differentiation (Fernández et al., 2017).

Estrogens are described as endocrine-disrupting chemicals (EDCs) with inherent potential adversative environmental and public health impacts (Fonseca et al., 2011). Estrogens exposure inhibits its metabolism (Sun et al., 2020c), sexual fitness (Dzieweczynski & Kane, 2017), and can induce vitellogenin in male fish (Selcer & Verbanic, 2014). Additionally, estrogen has a direct influence on fish biomass and has the potential to interfere with the aquatic food webs (Hallgren et al., 2014). Furthermore, a study has reported the detection of estrogens in the drinking water of China (Fan et al., 2013), suggesting a proper evaluation to determine their impact on humans. Therefore, several techniques were studied to remediate estrogens from environments, in principal forms of chemical (Li & Zhang, 2014), physical (Suri et al., 2010), and biological degradations (Kurisu et al., 2010).

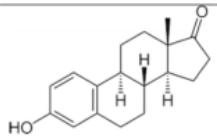
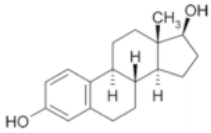
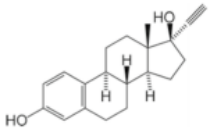
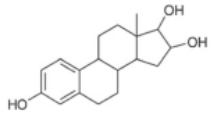
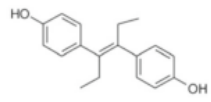
Among these, biological degradation is a promising technique to remediate various contaminants from the environment (Al Farraj et al., 2019a, 2019b, 2020; Hadibarata et al., 2018; Syafiuddin & Fulazzaky, 2021). Essentially, it is an eco-friendly approach in comparison to chemical degradation, while more economical than physical degradation (Eltoukhy et al., 2020; Syafiuddin et al., 2018, 2019, 2020). Even though advances have been made in the hybridization of physical and chemical techniques, such as photodegradation, they are complicated and not practically convenient. Photodegradation needs catalysts and light energy for the remediation process (Kwarciak-Kozłowska, 2019). Hence, the use of biological methods is seemed to be a safe procedure for the degradation of estrogens from the water since it circumvents the complicated procedure while free of toxic materials use.

Considering the significant of this topic, this paper aims to critically review the capability of bacteria, fungi, and algae for sustainable bioremediation of emerging contaminants in the forms of natural and synthetic estrogens from the water environment. This paper highlights several relevant aspects, including removal performance, advantages and disadvantages, kinetic behaviors, transformation of natural and synthetic estrogens, and some future perspectives as well as suggestions for improvement. This paper is useful for comprehensive remediation techniques design in employing the use of microorganisms.

2 Characteristics of Estrogen

The chemical structures and physio-chemical characteristics of estrogens are tabulated in Table 1. The chemical structure of natural estrogen contains the C18 steroidal group (Adeel et al., 2017). The characteristics of both natural and synthetic estrogens can be defined by molecular weight (MW), water solubility, log K_{ow}, and H-bond capacity, which provide noteworthy information for determining the biodegradability. Higher MW has a lower removal potency (Hom-Diaz et al., 2015). Besides MW, the molecular structure has possible effects on the biodegradation rate (Zhang et al., 2011). Depending on the substituents, it can influence the degradation efficiency. The water solubility of estrogen can influence their behaviors in water systems (Shareef et al., 2006). Water solubility is influenced by several parameters, which include pH and temperature (Ben Fredj et al., 2015). A higher solubility produces a greater concentration of free estrogen molecules in water for absorption (Eibes et al., 2011; Lai et al., 2000; Schäfer et al., 2011). The coefficient K_{ow} is a measure of hormone hydrophilicity/lipophilicity (Cumming & Rücker, 2017). The positive value of log K_{ow} reflects the hydrophobic characteristic, with larger value describing greater hydrophobicity. Molecules with a low or negative value of K_{ow} are frequently prescribed as polar, although there is no direct relation between K_{ow} and the charge distribution in the molecule.

Table 1 Chemical structures and psycho-chemical properties of estrogen

Type of estrogen	Structure	Molecular weight (Da)	Water solubility (mg/L at 20 °C)	log K_{ow}	H-bond capacity	Reference
E1		270.37	13	3.43	Strong OH donor and acceptor; Strong O acceptor; weak π acceptor (benzene)	Eibes et al. (2011), Lai et al. (2000), Schäfer et al. (2011)
E2		272.39	13	3.94	Strong OH donor and acceptor; weak π acceptor (benzene)	Eibes et al. (2011), Lai et al. (2000), Schäfer et al. (2011)
EE2		296.40	4.8	4.15	Strong OH donor and acceptor; weak π acceptor (benzene)	Eibes et al. (2011), Lai et al. (2000), Schäfer et al. (2011)
E3		288.39	13	2.81	Strong OH donor and acceptor; weak π acceptor (benzene)	Ghasemi et al. (2017), Lai et al. (2000), Schäfer et al. (2011)
DES		268	12	5.07	Strong OH donor and acceptor; weak π acceptor (benzene)	Schäfer et al. (2011)

H-bonding plays an important role in estrogen transport in biological systems. Also, estrogen possesses a phenol group, which exhibits a rich electron. However, it has the potential to form the π–π bonding with electron-deficient phenyl groups of the polymers (Schäfer et al., 2011). The hydrogen-bonding ability of estrogens was discovered previously by Morishima et al. (2013). The study established that a water molecule prefers to form an H-bond in A-ring OH for estrone and β-estradiol. It is because the acidity of phenolic OH is higher than alcoholic OH. On the contrary, in E3, the water molecule desires to form an H-bond in D-ring due to a stable formation of the H-bonding ring structure system with two OH groups. Other studies had found the hydrogen-bonding abilities of EE2 (Jiang et al., 2017) and DES (Abou-Zeid & El-Mowafy, 2002), which confirmed that the phenolic hydroxyl and alcoholic hydroxyl compounds are required for degradation.

3 Removal of Estrogen Using Microorganisms

3.1 Removal by Bacteria

A series of studies on the degradation of estrogens by microorganisms is summarized in Table 2. Zhao et al. (2020) found that the *Serratia nematodiphila* DH-S01 strain degraded E1 at an elapsed time of 96 h. After 96 h, the estrone degradation rate reached 93.47%, which was a significant degradation of E1. Another study isolated a potential microbial strain (BH2-1) for the biodegradation of E1 (Pratush et al., 2020). The study exhibited that E1 could be degraded by 89.5% by strain BH2-1 in accordance with an exposure of 6 days of incubation in the MSM-E1 medium containing 1% NaCl at pH 6, and thus confirming an excellent E1-degrading potential. *Novosphingobium sp*. ARI-1 bacterium was also immobilized in calcium alginate (CA) (Liu et al., 2018a). Immobilized ARI-1 was used to remove estrogens from natural sewage and cow dung with a removal rate of 80.43% E1 from sewage containing 1.75 μg/L E1 within 7 days.

The removal efficiency of 95% steroid E2 by *Gordonia sp.* was witnessed at a temperature range of 10 and 40 °C with a complete degradation after 48 h (Liu et al., 2020). This finding implies that *Gordonia sp.* could degrade E2 over a very wide range of temperatures. E2 could also be degraded by the *Deinococcus actinosclerus* SJTR1 strain (Xiong et al., 2018). With a concentration of 10 mg/L, 90% of E2 was isolated from the wastewater with almost a full efficiency in 5 days, confirming the good efficacy of the strain in reducing E2. Additionally, Li et al. (2012b) showed E2 was degraded by *Stenotrophomonas maltophilia* ZL1 strain from a concentration of 3.3 to 0.02 mg/L in 16 h. After 36 h, E2 concentration remained stable with a degradation of nearly 100%.

Table 2 also presents a 100% E3 removal by immobilized *Novosphingobium sp.* ARI-1 from natural sewage and cow dung (Liu et al., 2018a), for initial concentrations of 1.52 μg/L and 0.66 mg/kg, respectively. The result ascertains the effectiveness of immobilized strain ARI-1 for E3 degradation from the environmental matrices. As an endocrine-disrupting compound, the degradation of E3 had been shown by an isolated *Pseudomonas putida* SJTE1 strain from an enrichment culture of sludge, displaying a full efficacy after 120 h from a concentration of 10 mg/L (Wang et al., 2019a). A different strategy to remove E3 was accomplished using a facultative anaerobic strain of iron-reducing bacteria (Ivanov et al., 2010). The study found that a 60% reduction of an initial concentration of 100 μg/L E3 was achieved, a performance of which remained after 15 days of batch cultivation. The study has showcased the ability of facultative anaerobic iron-reducing bacteria for E3 remediation from the rejected water in the municipal wastewater treatment plant.

Sedighi et al. (2019) studied the degradation of EE2 by *Enterobacter tabaci* and noticed almost 100% removal efficiency at initial concentrations of 0–4 mg/L in the aqueous phase after 96 h. Further, biodegradation of EE2 was observed using heterotrops bacteria (Larcher & Yargeau, 2013a). The bacteria were commonly presented in activated sludge. The results showed that *Rhodococcus rhodochrous* was the most successful with no visible EE2 after 48 h, indicating 100% removal of EE2. Other examined bacteria attained 21–61% of EE2 removal. Nonetheless, the study recommended the use of WW treatment/activated sludge strain. A similar study was performed using several bacteria (Larcher & Yargeau, 2013b). Besides using groups of bacteria, the study applied an ozone strategy to improve the removal treatment. The study reported two different conditions: with and without ozonation. Without

Table 2 Percentage of estrogen degradation by the microorganism

Estrogen	Organism	Degradation (%)	Reference
17β-estradiol	*Pycnoporus sp.*	>80%	Liu et al. (2016b)
17β-estradiol	Lignin peroxidase (LiP) from white-rot fungi, such as *Phanerochaete chrysosporium*	~100%	Mao et al. (2010)
17α-ethinylestradiol	*Agaricus bisporus* (stalk)	100%	Menk et al. (2019)
17α-ethinylestradiol	*Lentinula edodes* (stalk)	100%	Menk et al. (2019)
17α-ethinylestradiol	*Lentinula edodes* (substrat)	80%	Menk et al. (2019)
17β-estradiol	*Fusarium sp.* KY123915	92.5%	Wu et al. (2018)
Estrone, 17β-estradiol, estriol, 17α-ethinylestradiol	*Pleurotus ostreatus* HK 35	>90%	Křesinová et al. (2018)
17β-estradiol	*Bjerkandera adusta*	88%	Loffredo et al. (2016)
17β-estradiol	*Irpex lacteus*	89%	Loffredo et al. (2016)
17α-ethinylestradiol	*Pleurotus ostreatus*	About 90%	Křesinová et al. (2018)
Estrone	*Myceliophthora thermophila*	Up to 95%	Lloret et al. (2012)
Estrone, 17β-estradiol, 17α-ethinylestradiol	*Myceliophthora thermophila*	60 to 90%	Lloret et al. (2012)
17-α-ethinylestradiol	*Pycnoporus sanguineus*	96.5%	Golveia et al. (2018)
17β-estradiol	*Novosphingobium sp*	52.1–100%	Li et al. (2020)
17β-estradiol	*Gordonia sp.*	Almost 100%	Liu et al. (2020)
17β-estradiol	*Deinococcus actinosclerus* SJTR1	Nearly 90%	Xiong et al. (2018)
17β-estradiol	*Sphingomonas* strain KC8	62%	Roh and Chu (2010)
17β-estradiol	*Lysinibacillus sphaericus*	About 97%	Wang et al. (2020b)
17β-estradiol	*Stenotrophomonas maltophilia* SJTH1	90%	Xiong et al. (2018)
17β-estradiol	*Stenotrophomonas maltophilia* SJTL3	Over 90%	Xiong et al. (2018)
Estrone	Microbial strain BH2-1	89.5%	Pratush et al. (2020)
Estrone	*Serratia nematodiphila* DH-S01	93.47%	Zhao et al. (2020)
17β-estradiol	*Serratia nematodiphila* DH-S01	93.2%	Zhao et al. (2020)
17β-estradiol	*Shewanella oneidensis* MR-1	95%	Dai et al. (2019)
17α-ethinylestradiol	Enterobacter tabaci	Almost 100%	Sedighi et al. (2019)
17β-estradiol	*Chlorella algae*	92%	Huang et al. (2019)
17β-estradiol	*Nannochloris sp.*	60%	Bai and Acharya (2019)
17α-ethinylestradiol	*Nannochloris sp.*	60%	Bai and Acharya (2019)
Estrone	*Nannochloris sp.*	29%	Bai and Acharya (2019)
17β-estradiol	*Microcystis aeruginosa*	73–85%	Bai et al. (2019)
17β-estradiol	*Pseudomonas putida*	90%	Wang et al. (2019a)
Estriol	*Pseudomonas putida*	90%	Wang et al. (2019a)
Estrone	*Scenedesmus obliquus*	91%	Ruksrithong and Phattarapattamawong (2019)
17β-estradiol	*Scenedesmus obliquus*	99%	Ruksrithong and Phattarapattamawong (2019)
Estrone	*Chlorella vulgaris*	52%	Ruksrithong and Phattarapattamawong (2019)
17β-estradiol	*Chlorella vulgaris*	99%	Ruksrithong and Phattarapattamawong (2019)
Estrone	*Spirulina* CPCC-695	53.7–94.5%	Sami and Fatma (2019)
Estrone	*Novosphingobium* sp.	80.43%	Liu et al. (2018a)

Table 2 (continued)

Estrogen	Organism	Degradation (%)	Reference
17β-estradiol	*Novosphingobium* sp	94.76%	Liu et al. (2018a)
Estriol	*Novosphingobium* sp	100%	Liu et al. (2018a)
17β-estradiol	LM1 bacteria isolated from Manure	77%	Li et al. (2018)
17β-estradiol	LY1 bacteria isolated from Manure	68%	Li et al. (2018)
17β-estradiol	*Raphidocelis subcapitata*	74.6–82.0%	Liu et al. (2018a)
Diethylstilbestrol	*Raphidocelis subcapitata*	54.1–89.9%	Liu et al. (2018a)
17β-estradiol	*Selenastrum capricornutum*	93.9%	Wang et al. (2017)
17α-ethinylestradiol	*Selenastrum capricornutum*	75.3%	Wang et al. (2017)
Diethylstilbestrol	*Serratia sp.*	90.0–96.7%	Ling et al. (2016)
17β-estradiol	*Rhodococcus sp.* JX-2	64%	Liu et al. (2016a)
17β-estradiol	*Rhodococcus sp.* JX-2	81%	Liu et al. (2016a)
17β-estradiol	*Sphingomonas sp.*	94%	Ma et al. (2016)
17α-ethinylestradiol	*Desmodesmus subspicatus*	68%	
17β-estradiol	*Stenotrophomonas maltophilia*	Nearly 100%	Li et al. (2012b)
17α-ethinylestradiol	α-, β-, and γ-proteobacteria	90%	Pauwels et al. (2008)
Estrone	α-, β-, and γ-proteobacteria	>99%	Pauwels et al. (2008)
Estriol	α-, β-, and γ-proteobacteria	>99%	Pauwels et al. (2008)
Diethylstilbestrol	*Pseudomonas sp.* strain J51	80%	Zhang et al. (2013)
Estrone	A mixture of pure cultures of six different algae	95%	Shi et al. (2010)
Estrone	A mixture of pure cultures of six different algae	50%	Shi et al. (2010)
17β-estradiol	Immobilized *Desmodesmus sp.*WR1	85–99%	Wang et al. (2020a)
Estrone	*Scenedesmus dimorphus*	85%	Zhang et al. (2014)
17β-estradiol and estriol	*Scenedesmus dimorphus*	95%	Zhang et al. (2014)
Estrone	*H. pluvialis*, *S. capricornutum*, and *S. quadricauda*	97%, 80%, and 97%	Wang et al. (2019b)
17β-estradiol	*H. pluvialis*, *S. capricornutum*, *S. quadricauda*, and *C. vulgaris*	100%	Wang et al. (2019b)
17α-ethynylestradiol	*H. pluvialis* and *S. quadricauda*	85%	Wang et al. (2019b)
Estriol	Anaerobic strain of iron-reducing bacteria	60%	Ivanov et al. (2010)
17α-ethynylestradiol	*R. rhodochrous*	100%	Larcher and Yargeau (2013a)
17β-estradiol, 17α-ethinyl estradiol and estriol	*Moniliophthora roreri* (Mrl2)	>90%	Bronikowski et al. (2017)
17α-ethynylestradiol	Bacterial mixtures	40–43%	Larcher and Yargeau (2013a)

ozonation, the proposed bacterial mixture can remove EE2 after 300 h with removal efficiencies ranging from 42 to 43%. However, there was no an increase in the removal efficiency after ozonation. A study on immobilized *Serratia sp.* was performed to degrade DES from sewage outfalls (Ling et al., 2016). The efficiencies of DES removal were 90.0%, 96.0%, and 96.7%, respectively, for initial concentrations of 40.01, 37.90, and 33.52 μg/L sewage outfalls. It provides an alternative technique for estrogen removal from the polluted water systems.

A similar study by Ke et al. (2007) on E1 degradation by CYH strain bacteria described that the degradation followed the Michelis-Menten kinetics. *CYH* strain was identified by 95% accuracy as *Sphingomonas*. The degradation of E1 by CYH was under anoxic conditions. Under aerobic conditions, the degradation followed linear kinetics ($R^2 > 0.9$). The study

suggested that different degradation mechanisms under aerobic and anoxic conditions involved different enzymes. Having seen these outcomes, further works are suggested to inspect the degradation mechanism of E1 at different condition such as anoxic.

Kinetic degradation behaviors of E2 by bacterial co-culture isolated from manure at different initial concentrations were recorded by Li et al. (2018). The kinetic degradation was assessed using the first-order kinetics. At various initial concentrations from 1 to 20 mg/L and in acetone-free mineral salt medium, the study presented $t_{1/2}$ values from 1.004 to 1.947 days and first-order rate constant (k) values from 0.690 ± 0.029 to 0.356 ± 0.012. However, the degradation showed the highest R^2 value (0.995) at 1 mg/L, implying the best fitting performance. Moreover, the study identified that most E2 at the initial concentration of less than 10 mg/L experienced rapid degradation (3 days incubation). At an increased initial concentration of up to 20 mg/L, extensive time was needed for the degradation by bacterial co-culture. Thus, further research is recommended to investigate the underlying mechanisms that affect significantly the bacterial growth and E2 degradation in the mineral salt medium.

Another biodegradation of E3 using dehydrogenase 17β-HSDx from *Rhodococcus sp.* was assessed (Ye et al., 2019). It was found that 17β-HSDx had a strong recommendation to transform E3. 17β-HSDx had dehydrogenation ability to transform E3 to 16-hydroxestrone. Nevertheless, the structure of 17β-HSD from bacteria was unconfirmed. Therefore, further study in the future needs to analyze the structure of 17β-HSDx and comprehend its catalytic mechanism. Carr et al. (2011) studied the microbially mediated degradation of E3 under aerobic and anaerobic conditions in the soil, finding 0.7- and 1.7-day half-lives for the aerobic-anaerobic conditions, respectively. Hence, E3 exhibited slightly shorter half-lives under aerobic conditions than under anaerobic conditions. However, all treatment regressions were significantly different ($p < 0.001$) by ANOVA analysis. Nonetheless, the specific bacteria community was not established. Therefore, further study is needed to investigate the bacteria strain in soil. The kinetic bioremoval of E3 was investigated using immobilized ARI-1 by Liu et al. (2018a). The study presented that kinetic removal was suitably fitted with the first-order reaction equation by $19.526e^{-0.552}$ and the half-life of E3 was 1.25 days. Moreover, the correlation coefficient (R^2) was more than 0.95. The computed R^2 value specified that E3 in culture medium was effectively degraded by immobilized *ARI-1*. However, there is a need to identify the sorption and desorption of E3 and enzymes in gel beads in the future study.

Besides using laccase from microfungal, EE2 also could be degraded using *Enterobacter tabaci* (Sedighi et al., 2019). The kinetic study adapted the Monod and allosteric sigmoidal models. Using Monod model, R^2 was 0.98. The constants of the model were the maximum specific degradation rate (q_m) = 3.527 and the half velocity constant (K_s) = 7.574. Using the allosteric sigmoidal model, R^2 was 0.99. The constants of the model were characterized by $q_m = 1.668$, $K_s = 7.282$, and the hill slope (h) = 2.207. Moreover, the study confirmed that *Enterobacter tabaci* could be revealed as a novel strain for biodegradation of EE2 estrogenic compounds.

The advantages and disadvantages of the use of bacteria for biodegradation of estrogens are noted in Table 3. Their corresponding details are discussed in the following. Firstly, bacteria for estrogen biodegradation can be performed under various conditions. In addition, *Stenotrophomonas maltophilia* SJTH1 degraded E2 efficiently, either in saline-, heavy metal-, or surfactant-contained conditions (Xiong et al., 2020b). Then, the ability to oxidize has been one of the useful behaviors possessed by bacteria. EE2 could be removed using ammonia-oxidizing bacterial (AOB) culture (Khunjar et al., 2011). Further, bacteria are adaptable at low temperatures. *Pseudomonas psychrophila* HA-4 was cold-adaptable to remove 34.3% pharmaceutical compound (SMX) after 192 h of cultivation (Jiang et al., 2014). Also, bacteria can be greatly tolerant at high pollutant concentrations. High tolerance of *Pseudomonas putida* had been observed to remove high organic compound (phenol) concentrations within 24 h (Al-Zuhair & El-Naas, 2011). Besides, bacterial degradation was a cost-effective and eco-friendly method than those of physical and chemical (Eltoukhy et al., 2020).

The use of *Pseudomonas putida* with rhamnolipid or ultrasonication requires additional strategies, which could increase the biodegradation rate (Chen et al., 2017a). When the retention time was increased to 72 h, the effluent concentrations of E1 and E2 were not detectable, hence suggesting that higher retention time is needed by bacterial biodegradation of

Table 3 Advantages and disadvantages of biodegradation using microorganisms

Organism	Advantage	Disadvantage	Reference
Bacteria	• Functional under various conditions • Able to oxidize • Adaptable at low temperatures • Tolerant at high pollutant concentrations • Cost-effective and eco-friendly	• Need additional strategies • Long retention time • Need additional carbon source • Need immobilization • Low removal percentage	Al-Zuhair and El-Naas (2011), Chen et al. (2017a), Eltoukhy et al. (2020), Jiang et al. (2014), Khunjar et al. (2011), Li et al. (2017, 2018), Liu et al. (2018a), Ma et al. (2016), Muller et al. (2010), Xiong et al. (2020a)
Fungi	• Able to oxidize • Safe and low-cost • Produce diverse coupling product • Efficient production of biodiesel • Tolerant under salinity condition	• Need nutrient addition • Could not remove toxicity • Long depletion period • Need immobilization	Badia-Fabregat et al. (2015), Daâssi et al. (2016), de Freitas et al. (2017), Dzionek et al. (2018), Lloret et al. (2010), Rodríguez-Rodríguez et al. (2010), Różalska et al. (2015), Sun et al. (2020b), Vasiliadou et al. (2016)
Algae	• Tolerant to environmental changes • Simultaneous generation of biofuel feedstock • Eco-friendly • Observed under anaerobic condition • Safe	• Need chlorophyll and carotenoids • Require nutrient for growth and biodegradation • Low removal rate • Need carbon source • Time-consuming	Bai and Acharya (2019), Hom-Diaz et al. (2015), Huang et al. (2019), Ji et al. (2014), Kozlova et al. (2020), Sami et al. (2020), Shi et al. (2010), Vo et al. (2020), Wang et al. (2020a)

estrogen (Ma et al., 2016). The specific degradation rates for E1 and EE2 using mixed bacterial cultures were seven and twenty times faster when these hormones were supplied as the only carbon source, but the degradation rate with or without carbon source was similar in the case of E2 (Muller et al., 2010). Immobilization is required in some cases for the bacterial reduction of estrogens, for instance, *Novosphingobium sp.* ARI-1 was immobilized in calcium alginate (CA) to enhance the environmental adaptability of bacteria at various temperatures and pH values (Liu et al., 2018a). In some studies, a low percentage of estrogen removal is seen. Strain E2S exhibited merely 66.4% degradation efficiency after 7 days (Li et al., 2017).

3.2 Removal by Fungi

A 95% performance rate of the bio-removal of E1 by laccase from *Myceliophthora thermophila* is noticed in Table 2 (Lloret et al., 2012). The study was conducted at the optimum oxygenation conditions (pulses every 30 min) and inspected at a higher hydraulic residence time of 4 h (feed addition rate of 1 mg/L h). An identical study using laccase from *Myceliophthora thermophila* found that 60 to 90% E1 could be degraded within 8 h under Lac 2000 U/L synthetic and natural mediator conditions (Lloret et al., 2010). Employing white-rot fungus *Pleurotus ostreatus HK 35* for E1 degradation, Křesinová et al. (2018) revealed under the laboratory model that the attained efficiency was greater than 90% within 12 days. Besides, their results disclosed that the fungus functioned without negative effects on the degradation performance and can be carried out in the presence of bacterial microflora in the WWTP.

From the sample collected from a wastewater treatment plant (WWTP) in Xi'an, Wu et al. (2018) showed a high reduction rate of 92.5% of E2 within 48 h by *Fusarium sp.* KY123915 when examined in the optimal pH (6) and temperature (30 °C). The degradation of E2 was also observed using Lignin peroxidase (LiP) as excreted by certain lignin-degrading fungi, such as *Phanerochaete chrysosporium* (Mao et al., 2010). The study showed the removal efficiency was nearly 100% with a recommendation that LiP-mediated oxidative coupling reactions had great potential in the wastewater treatment of E2 removal and estrogenicity. Furthermore, the study proposed possible progress by the coupling of E2 via covalent bonding among two E2 radicals at their unsubstituted carbons in phenolic rings. A study by Loffredo et al. (2016) had shown the exploitation of E2 biodegradation from the municipal landfill leachate (MuLL) using *Bjerkandera adusta*. The average percentage (after 15 days) of degraded E2 was 88%, suggesting

that E2 in contaminated adsorbents could be degraded by the ligninolytic fungi, *Bjerkandera adusta*.

Investigation on biodegradation of E3 by cultivated *Pleurotus ostreatus* HK 35 by Křesinová et al. (2018) displayed a 90% removal efficiency within 12 days as listed in Table 2. The degradation efficiency was inspected under laboratory model conditions, which portrayed the ability *of Pleurotus ostreatus* to treat E3 pollutants in the wastewater. Another strategy to remediate E3 from wastewater was by adopting laccase of fungal from *Moniliophthora roreri* (Mrl2) (Bronikowski et al., 2017). Under controlled neutral pH, the strategy employed Mrl2 within 30 min and showed a reduction of more than 90% E3. Hence, Mrl2 could degrade E3 as EDCs. Complete removal of EE2 by *Pleurotus ostreatus* was reported (Křesinová et al., 2012b). The study showed that EE2 can be degraded by the proposed organism by 90%. EE2 was completely removed from an amount of 200 µg in 20 mL liquid complex and mineral media in 3 and 14 days, respectively. A similar study had evaluated the biosorption efficiency of shiitake (*Lentinula edodes*) on EE2-contaminated water (Menk, 2019). In EE2 biosorption, shiitake stalk showed an ability to remove 100% in 20 min. On the other hand, the shiitake substrate exhibited 80% removal. This shows that shiitake stalk had the best results for the degradation of EE2. However, neither the stalk nor the substrate demonstrated the ability to remediate EE2. Nevertheless, the use of mushroom waste could reduce environmental impact and increase product value.

Another bioremediation strategy of EE2 was promoted using *Pycnoporus sanguineus* laccase (Golveia et al., 2018). *Pycnoporus sanguineus* ATCC 4518 was obtained from the Andre Tosello Foundation in Campinas, Sao Paulo, Brazil. The study used Theobroma grandiflorum residue as a laccase inducer. For bioremediation, Lac enzymatic extract was put in an EE2 solution (10 mg mL^{-1}). The study showed that 86% of EE2 removal was achieved after 4 h. After 8 h of reaction, 96.5% of EE2 was detached. Moreover, *Pycnoporus sanguineus* Lac was able to bioremediate EE2 in wastewater.

The bio-removal behavior of E1 by fungal *Trametes versicolor* laccase was mathematically identified by Auriol et al. (2008). The study presented a pseudo-first-order dependence on E1 concentration ($R^2 > 0.96$). Furthermore, it was characterized by the Michaelis–Menten equation that the Michaelis constant (K_M) was 3.40 representing the affinity of the enzyme to its substrate. Lower K_M value has a higher affinity. Also, K_{cat} value, which corresponds to the oxidation rate, was 0.01. Sun et al. (2020b) assessed using the Pseudo-first-order model the degradation of E2 by *Trametes hirsuta* La-7 with high laccase-productivity (CC-ThLac). They observed R^2 values of 0.9913, 0.9875, and 0.9950 at pH 4, 5, and 6, respectively. This displays that at pH 6.0, the highest R^2 value (0.9950) was obtained. The highest value indicates the best fit between the model to the experimental data. Moreover, the kinetic constant values, k and $t_{1/2}$, for 5 U·mL^{-1} CC-ThLac were 0.027–0.055 min^{-1} and 12.67–25.86 min^{-1} with pH values ranging from 4 to 6, respectively.

EE2 degradation observed using laccase from *Trametes versicolor* was characterized using the pseudo-first-order expression, yielding various k values of 0.0168, 0.0327, 0.0273, and 0.0079 at pH 4, 5, 6, and 7, respectively (Sun et al., 2020a). Moreover, the half-live values were 41.26, 21.20, 25.39, and 87.74 (pH 4–6). The study showed that all R^2 values were higher than 0.97. The highest R^2 value achieved was 0.9927 at pH 7. Therefore, the study demonstrated that fungal laccase was able to effectively catalyze the removal of EE2. EE2 kinetic degradation using laccase from *Trametes versicolor* was assessed using the Michaelis–Menten equation and pseudo-first-order by Auriol et al. (2008). The study obtained a K_M of 3.40 µM. K_M values describe the affinity of the enzyme to its substrate. Lower K_M value has a higher affinity. Moreover, K_{cat} as another kinetic parameter was 0.01 s^{-1}. Another kinetic parameter, k_{catto}-K_M ratio, was computed as 2.99×10^3. It reflects the catalytic efficiency. A higher ratio symbolizes more efficiency for estrogen removal. Furthermore, laccase catalyzed systems displayed closely a pseudo-first-order expression. It attained R^2 of more than 0.96. Additionally, the study also confirmed that the enzymatic treatment by laccase from *Trametes versicolor* was efficient in eliminating the estrogenic activity of the steroid estrogens.

The advantages and disadvantages of the use of fungi for biodegradation of estrogens can be seen in Table 3. The ability to oxidize EDCs by different fungal laccases was observed by Daâssi et al. (2016). Moreover, the use of *Pleurotus ostreatus* and *Pleurotus pulmonarius* to degrade EDCs is a safe and economic alternative to the widely used chemical

processes (de Freitas et al., 2017). Also, fungi can produce a diverse coupling product. E2 was step-polymerized to produce various oligomers by *Trametes hirsuta* La-7 via a radical coupling mechanism (Sun et al., 2020b). For eco-friendly energy generation, the efficient production of biodiesel was achieved from the residual fungal mass (Vasiliadou et al., 2016). Last but not least, the good tolerance under salinity condition was seen for the EE2 subsequent decrease in samples with or without salinity (Różalska et al., 2015).

In terms of disadvantage, some fungi need mediators, for example, *Myceliophthora thermophila* needed compounds with higher redox potentials like non-phenolic substances to degrade estrogens (Lloret et al., 2010). The mediators are, however, highly costly and often not environmentally friendly. Moreover, it was witnessed that *Pleurotus pulmonarius* laccase could not remove EDCs toxicity because its metabolite was as toxic as the parent compound itself (de Freitas et al., 2017). Further, the nutrient addition was needed to improve the removal in reverse osmosis concentrate (ROC) wastewater by *Trametes versicolor* fungal treatment (Badia-Fabregat et al., 2015). Also, a long depletion period is one of the fungi limitations in the biodegradation event. Complete depletion of EDCs was achieved only after 72 h (Rodríguez-Rodríguez et al., 2010). Also, an extra immobilization process of *Planococcus sp.* S5 strain cells was required to degrade 6, 9, 12, or 15 mg/L of pharmaceuticals (naproxen) faster than the free cells (Dzionek et al., 2018).

3.3 Removal by Algae

An investigation of E1 removal by *Scenedesmus obliquus* was carried out (Ruksrithong & Phattarapattamawong, 2019). The initial concentration of *Scenedesmus obliquus* was 100 mg/L as dried weight ($mgDW{\cdot}L^{-1}$). *S. obliquus* rapidly reduced E1 concentrations from 5 to 2 µg/L. The study showed that *Scenedesmus obliquus* reached up to a 91% reduction of E1. Furthermore, the study confirmed that microalgae cultivation of *Scenedesmus obliquus* could decrease E1 in synthetic piggery wastewater. Another species of *Scenedesmus*, *Scenedesmus dimorphus*, was used by Zhang et al. (2014) to evaluate the E1 degradation. The removal efficiency was 85% for estrone over 8 days. Using microalgae, a similar study of E1 degradation by *Haematococcus pluvialis*, *Selenastrum capricornutum*, and *Scenedesmus quadricauda* was considered (Wang et al., 2019b). The study showed that using *Selenastrum capricornutum* has the lowest removal efficiency (80%) than *Haematococcus pluvialis* (97%) and *Scenedesmus quadricauda* (97%). However, the findings suggested that microalgae could be supported in WWTPs as the progressive treatment of the E1 removal.

Huang et al. (2019) examined E2 degradation by *chlorella* algae via laboratory experiments. As seen in Table 2, the removal efficiency by chlorella was up to 92% after 10 days. The investigation employed E2 initial concentrations of 0.5 mg/L. It was declared that the presence of chlorella algae could play a role as a bioremediation agent for E2 removal in the aquatic environment. A study by Ruksrithong and Phattarapattamawong (2019) had shown that *chlorella vulgaris* could remove 99% E2 from synthetic wastewater. Besides *chlorella vulgaris*, the study investigated *Scenedesmus obliquus* to remove E2, both of which were conducted under steady-state conditions.

Wang et al. (2020a) observed from the wastewater treatment using the immobilized microalgae led to E2 removal efficiencies of 85–99%. The study was conducted with immobilized functional microalgae under domestic wastewater. Concentration above 200 beads/mL was suggested as an effective and safe to degrade the E2-contaminated wastewater. Other algae had disclosed also better remediation efficiencies. It was observed that several algae as considered by Wang et al. (2019b) showed 100% removal efficiencies for E2 degradation. The study found that substantial estrogenic activity reductions occurred after biotransformation by the four common microalgae. The study demonstrated also that widely living microalgae could degrade E2 as emerging pollutants in wastewater treatment plants (WWTPs).

Removal of EE2 by *Selenastrum capricornutum* had been investigated by Wang et al. (2017) as summarized in Table 2. The study employed *Selenastrum capricornutum* in SE medium cultivation. The biological removal of EE2 was found at 75.3% after 7 days. The result showed that *Selenastrum capricornutum* was capable to remove EE2 as a synthetic estrogen. Other microalgae, *Haematococcus pluvialis* and *Scenedesmus quadricauda*, were used also for EE2 removal (Wang et al., 2019b). The capability of the microalgae for EE2 removal

was observed for 40 days. The findings verified that the wild living microalgae could degrade EE2 as the emerging pollutants such that the study advised that microalgae could be a development treatment of WWTP to remove estrogens. EE2 degradation by the freshwater green alga *Desmodesmus subspicatus* was investigated (Maes et al., 2014). The investigation presented that up to 68% of the test compound was removed by *Desmodesmus subspicatus*. The study used algae concentrations of up to 2200 L/kg wet weight. Remarkably, *Desmodesmus subspicatus* brominated EE2 when bromide was available in the medium. The presence of bromine has arisen abiotically although this was not contributed by algae, suggesting a further inspection is needed. In addition, further test on lower EE2 concentration, a representative organic matrix, and different algae species are important for further exploration of algae efficacy in future investigation. A study on the bioremediation of DES by *Raphidocelis subcapitata* was carried out by Liu et al. (2018b). It was found that the biodegradation of DES by *Raphidocelis subcapitata* was noticed for 96 h with removal achievements of 89.9%, 73.4%, and 54.1% at different concentrations of 0.1, 0.5, and 1.5 mg/L, respectively. In addition, *Raphidocelis subcapitata* has the potential for the bioremediation of DES in water treatment.

A study on E1 removal by *Scenedesmus* and *Chlorella* was conducted by Ruksrithong and Phattarapattamawong (2019). The study evaluated kinetic behaviors using the first-order kinetics model. It was found that the coefficient of determination (R^2) predicted by linear fitting of experimental data collected at various times for *Scenedesmus* (0.937) was higher than by *Chlorella* (0.925). This indicates that a better linear fitting is offered by *Scenedesmus*. The adsorption capacity at equilibrium (q_e) value by *Chlorella* (0.0053) was observed to be lower than by *Scenedesmus* (0.0063). The pseudo-second-order rate constant (k_2) by *Scenedesmus* was higher (263.31) than by *Chlorella* (240.91). This reflects a lower affinity of *Chlorella* for E1 absorption.

Additionally, the Pseudo-first-order model was adopted for describing the degradation of E2 by microalgae *chlorella* (Huang et al., 2019) and *Scenedesmus dimorphus* (Zhang et al., 2014). Using chlorella, the degradation rate constants, k_s, of E2 at 1 mg/L and 0.5 mg/L were 0.11 and 0.35, respectively. Furthermore, R^2 at 1 mg/L and 0.5 mg/L were obtained as 0.70 and 0.83, respectively. *Scenedesmus dimorphus* forecasting showed a higher R^2 value (0.99). However, these two studies showed quite effective behavior for removing the emerging contaminants from WWTP.

Solé and Matamoros (2016) examined EE2 degradation using *Selenastrum capricornutum* with the pseudo-first-order equation, employing microalgae and microalgae beads. Using microalgae, the removal rate was obtained as 0.316 ± 0.021. Moreover, the half-life was 2.2 ± 0.2. The removal was $97 \pm 1\%$ with an R^2 value of 0.952 ± 0.030. Using microalgae beads, the removal rate and half-life were 0.0338 ± 0.039 and 2.1 ± 0.2. The study showed that using microalgae beads, higher $R^2 = 0.955 \pm 0.012$ could be achieved than using microalgae. Further, the study confirmed that the use of microalgae technology is a suitable alternative for EE2 removal wastewater treatment.

The advantages and disadvantages of the use of algae for biodegradation of estrogens are listed in Table 3. *Scenedesmus quadricauda* was commonly employed because of its tolerance to environmental changes in an aquaponics system (Kozlova et al., 2020). Simultaneous generation of biofuel feedstock had been observed raising the potential use of *Chlamydomonas mexicana* and *Chlorella vulgaris* microalgae for BPA biodegradation (Ji et al., 2014). Also, *Spirulina* CPCC-695 provided an eco-friendly approach to degrade estrone as endocrine-disrupting compounds (Sami et al., 2020). Using algae, E2 could be first oxidized to E1 under aerobic or anaerobic conditions, thus offering leniency in biodegradation operation (Shi et al., 2010). Further, a good safety advantage was found in immobilized functional microalgae at concentrations above 200 beads/mL as recommended in restoring E2-contaminated wastewater (Wang et al., 2020a).

The disadvantage of the adoption of fungi comes in the form of the need to additionally include chlorophyll and carotenoids to help to maintain intact cell membranes and for algae cell survival (Huang et al., 2019). Also, several nutrients (sodium nitrate, citric acid, ferric ammonium citrate, and ethylene diamine-tetra-acetic acid) were needed for algae growth and E2 degradation (Huang et al., 2019). EDCs such as E2, E1, EE2, and BPA

can be removed from the wastewater but only at fairly low rates (Bai & Acharya, 2019). Moreover, the addition of carbon sources for microalgae was required to remediate micropollutants using *Chlorella* sp. in wastewater (Vo et al., 2020). Furthermore, a time-consuming long period (> 7 days) was needed for EE2 to achieve high removal percentages (Hom-Diaz et al., 2015).

4 Estrogen Transformation Using Microorganisms

4.1 E1 Transformation

Transformation products in E1 degradation can be perceived by algae and bacteria strain (Kurisu et al., 2010; Wang et al., 2019b). As shown in Fig. 1, the

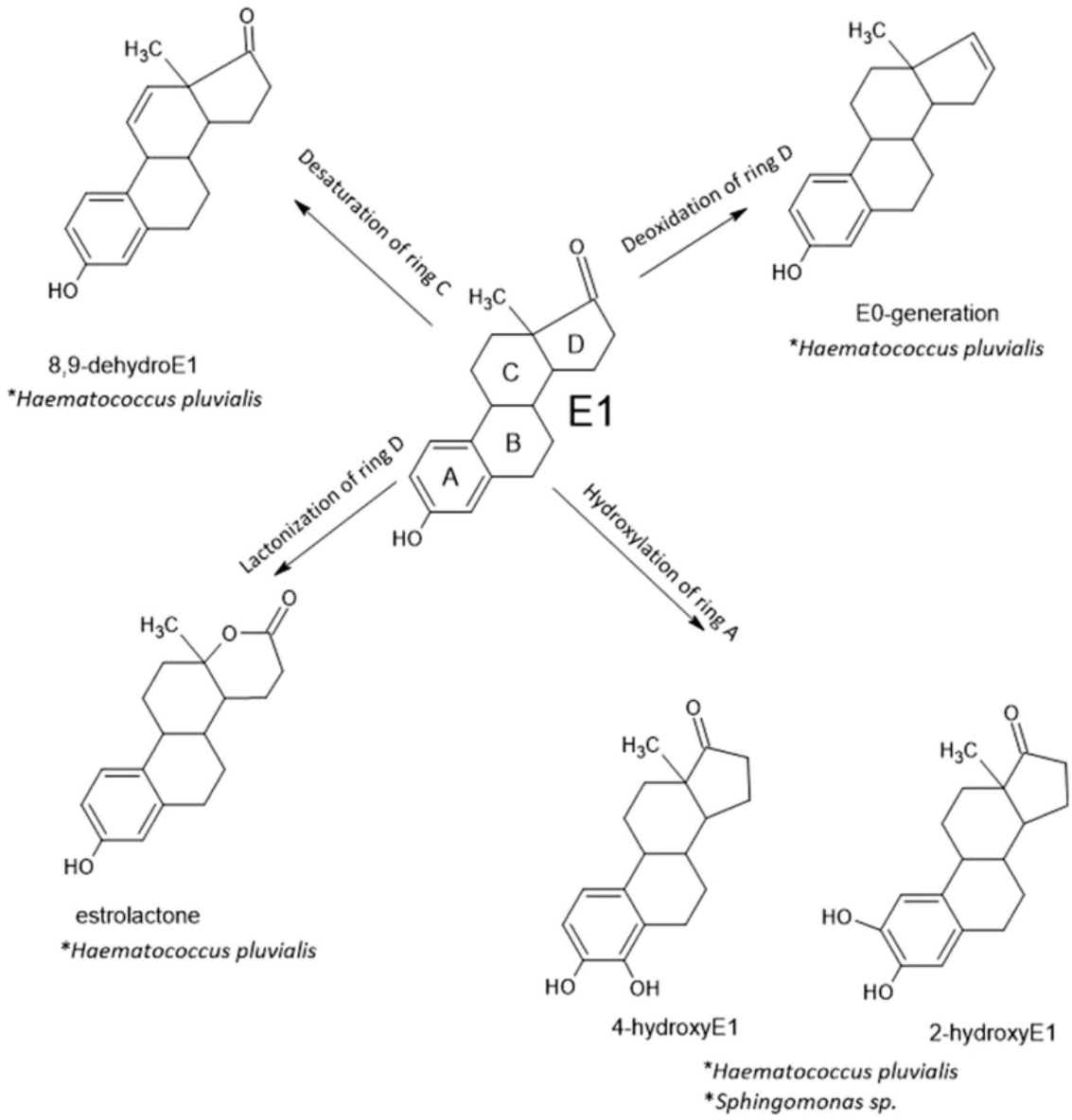


Fig. 1 Proposed biotransformation pathway of E1. It is noted that the mark (*) is the example of a biodegrading organism

first step of E1 degradation pathways can be classified into four groups: (a) deoxidation of ring D, (b) hydroxylation of ring A, (c) desaturation of ring C, and (d) lactonization of ring D (Wang et al., 2019b). Wang et al. (2019b) conducted a study on the degradation of E1 identified metabolites and the generation of E0 during the degradation in the culture of *Haematococcus pluvialis*. The study showed E0 has a lower estrogen activity compared to E1. The result of the E0 generation suggested that E1 was experiencing deoxidation at ring D. Several investigations on the metabolism of E1 degradation found the formation of hydroxylation products by *Haematococcus pluvialis* and *Sphingomonas sp.* microalgae strains (Kurisu et al., 2010; Wang et al., 2019b). 4-hydroxyE1 and 2-hydroxyE1 assumed to be intermediate metabolites were formed. Using microalgae, the study confirmed that this is followed by the reduction and methylation reaction (Wang et al., 2019b). Using the strain, the study confirmed that further degradation was via meta cleavage (Kurisu et al., 2010).

Using *Haematococcus pluvialis* for E1 degradation, the 8,9-dehydroE1 formation had been also inspected (Wang et al., 2019b). The transformation and accumulation were formed during the drop in E1. It was formed via ring C desaturation (Ma & Yates, 2017). A study on E1 degradation using *Haematococcus pluvialis* had exposed estrolactone as a metabolism product (Wang et al., 2019b). It was formed during the decrement of E1 detected via ring D lactonization (Yu et al., 2019).

4.2 E2 Transformation

Biotransformation of E2 can be performed by microorganisms, such as microalgae, microfungal, and bacteria. As seen in Fig. 2, the biotransformation of E2 is classified into several first steps, such as dehydrogenation-hydroxylation, hydroxylation, dehydrogenation, glycosylation, dehydration, and ketonization. The biotransformation of E2 by *Scenedesmus quadricauda* algae is presented in Fig. 2 (Wang et al., 2017). The study showed that microalgae could transform E2 to 2-hydroxyE1. This metabolites product was obtained via dehydrogenation and hydroxylation. Investigation of E2 degradation pathways by *Selenastrum capricornutum* algae was shown via hydroxylation of ring A (Hom-Diaz et al., 2015). There are two metabolite products. 2-hydroxyE2 and 4-hydroxyE2 were transformed as intermediate metabolite products during E2 degradation. Following the hydroxylation of ring A, hydroxylation of ring D could occur. Hydroxylation of ring D shows the possibility that the transformation can happen from E2 to E3 product. These metabolite products were found during E2 degradation by *Phanerochaete chrysosporium* microfungus (Zhou et al., 2015). A similar metabolites product was obtained by E2 degradation using *Scenedesmus quadricauda* algae (Wang et al., 2017).

Estrone as metabolites product of E2 degradation could be formed by 17β-hydroxysteroid dehydrogenase in *Rhodococcus sp.* (Ye et al., 2017). The intermediate metabolites product was exposed via dehydrogenation of ring D during E2 degradation. A similar result was reflected by E2 degraded using *Phanerochaete chrysosporium* microfungus (Zhou et al., 2015) and microalgae including *Selenastrum capricornutum* (Hom-Diaz et al., 2015), *Acinetobacter* (LM1), and *Pseudomonas* (LY1) (Li et al., 2018). Biodegradation of E2 by using *Desmodesmus sp.*WR1 microalgae was previously observed (Wang et al., 2020a). As shown in Fig. 2, metabolites product from E2 has potentially formed via glycosylation ring A. The formation product via glycosylation ring A was identified as 2-E2-glycoside. Degradation pathway of E2 had been also inspected using *Nitrisomonas europaea* by Nakai et al. (2011). Using *Nitrosomonas europaea*, E0 generation was transformed during E2 degradation. This derived product was determined via dehydration of ring D. Further, E0 was also degraded by *Nitrosomonas europaea* and its degradation rate was faster than E2. Metabolism pathway of E2 via ketonization of ring B was converted using *Stenotrophomonas maltophilia ZL1* by Li et al. (2012b). Figure 2 shows that E2 could be directly converted to keto-E1. Alternatively, E2 could also be degraded to E1 first before degrading to keto-E1. Furthermore, the study suggested that *Stenotrophomas maltophilia* ZL1 initiated E2 cleavage on ring D.

4.3 E3 Transformation

The behavior of E3 transformation by *Agromyces* (LHJ3) strain was identified by Ke et al. (2007). In aerobic cultures of *LHJ3*, a transient transformation product was detected with a MW of 286. The product was recognized as 16-hydroxyestrone (Fig. 3). It

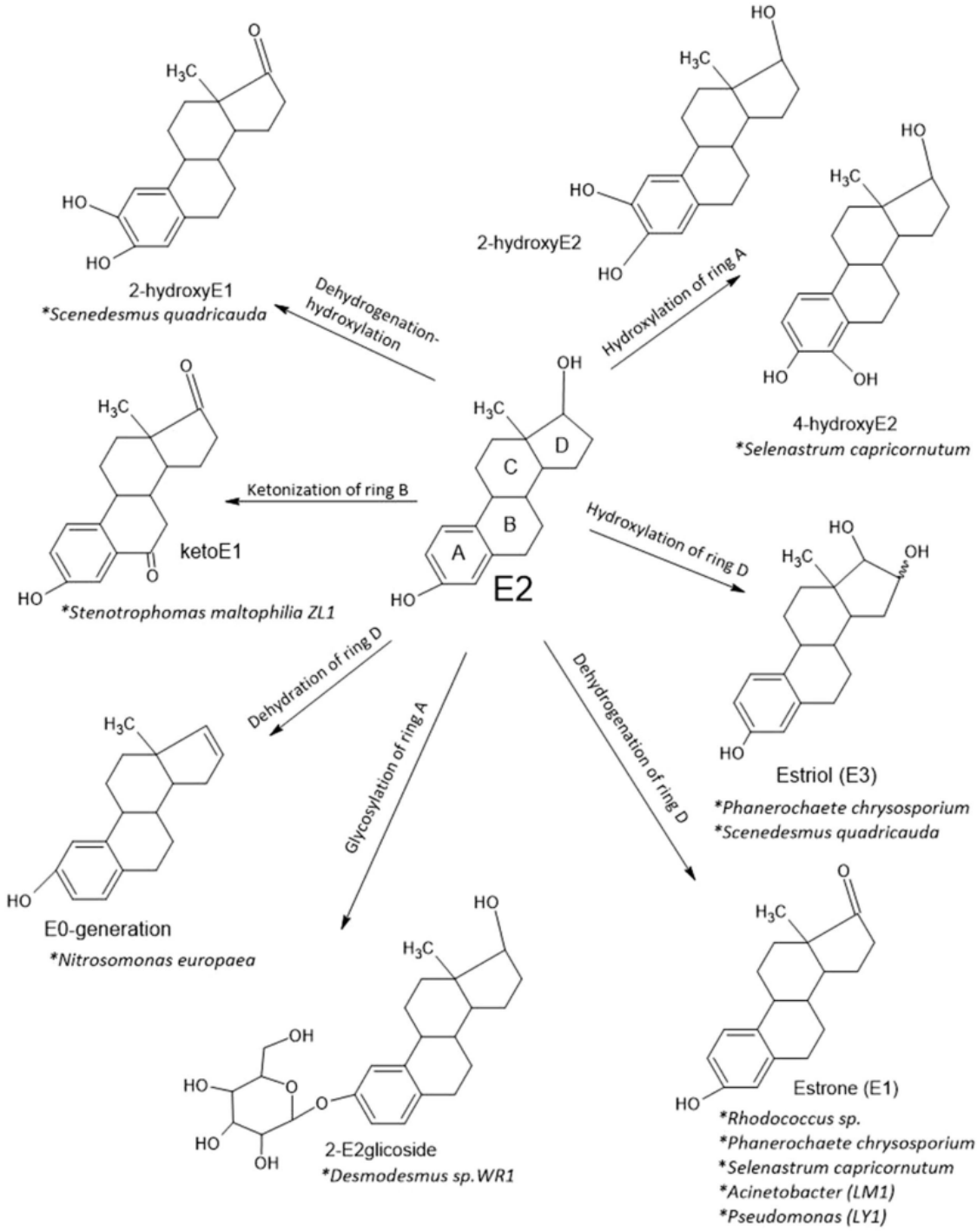


Fig. 2 Proposed biotransformation pathway of E2. It is noted that the mark (*) is the example of a biodegrading organism

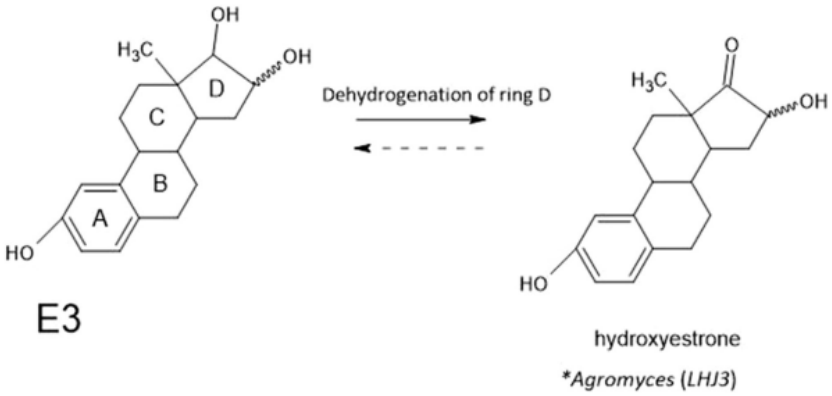


Fig. 3 Proposed biotransformation pathway of E3. It is noted that the mark (*) is the example of a biodegrading organism

was exposed via dehydrogenation of ring D. Also, E3 could be formed via hydroxylation. A similar study showed 17β-HSDx from *Rhodococcus sp.* P14 has dehydrogenation ability to transform E3 to 16-hydroxyestrone (Ye et al., 2019).

4.4 EE2 Transformation

Degradation pathways of EE2 can be prescribed by microalgae, microfungal, and bacteria strains. Figure 4 presents the first step of EE2 degradation pathways. The pathways can be identified in several groups, such as hydroxylation, dehydrogenation, ketonization, elimination, and glycosylation. EE2 transformation by microalgae *C. vulgaris* to convert EE2 to 4-OHEE2 was executed by Wang et al. (2019b). The detection of 4-OHEE2 was indicated by the hydroxylation of EE2 at ring A. Another study elsewhere using *Chlamydomonas reinhardtii* microalgae showed that hydroxylation at ring A could transform EE2 to 2-OHEE2 (Hom-Diaz et al., 2015). Besides, other studies showed that bacteria can transform EE2 into 4 OHEE2 (Khunjar et al., 2011; Ma et al., 2018). The degradation pathway of EE2 was assessed using *Pleurotus ostreatus* microfungus by Křesinová et al. (2012a). Figure 4 depicts 6-AHEE2 as a metabolism product. It was found via hydroxylation at ring B. A line of study had reflected a similar degradation product using *Chlamydomonas reinhardtii* microalgae (Hom-Diaz et al., 2015).

Using similar microfungus, the metabolism study of EE2 biodegradation was evaluated by *Pleurotus ostreatus* in Křesinová et al. (2012a). As seen in Fig. 4, the microfungus could transform EE2 to the 2-AHEE2 structure. The transformation was conducted via hydroxylation at ring C. With EE2 transformation via hydroxylation, EE2 shows the possibility also to transform via dehydrogenation. Dehydrogenation of EE2 by *Pleurotus ostreatus* could affect the EE2 structure (Křesinová et al., 2012a). Figure 4 shows the EE2 structure can be transformed via dehydrogenation at ring B. The structure could be transformed into 6,7-didehydroEE2. Besides affecting dehydrogenation at ring B, Křesinová et al. (2012b) exhibited estrogenic activity changes at ring C. As seen in Fig. 4, the study showed an effective dehydrogenation at ring C. Dehydrogenation at ring C changed EE2 into 9,11-didehydroEE2 formation.

The transformation of structure EE2 was also confirmed using the microbial community (Ma et al., 2018). It was observed that the proposed organism mixture transformed EE2 to E1 formation via dehydrogenation and oxidation on ring D. Investigation on EE2 degradation was inspected by *Trametes versicolor* laccase microfungus (Sun et al., 2020a). The study detected 3-ketoEE2 as a degradation product. It was found via ketonization at ring A. Similar study confirmed EE2 to the 3-ketoEE2 formation by *Scenedesmus quadricauda* (Della Greca et al., 2008). Degradation on EE2 structure was found using *Aspergillus sp.* (Różalska et al., 2015). The EE2 degradation pathway by *Aspergillus sp.* is presented in Fig. 4, illustrating EE2 structure transformation to E2 via elimination at ring D. Metabolite transformation of EE2 can be initiated by *Selenastrum capricornutum* (Della Greca et al., 2008). The transformation pathway was projected via glycosylation. As shown in Fig. 4, the metabolites product from EE2 was possibly transformed into EE2-glycoside ($R = R^1 = H$), 3-β-d-glucopyranosyl-2-hydroxyEE2 (R = OH,

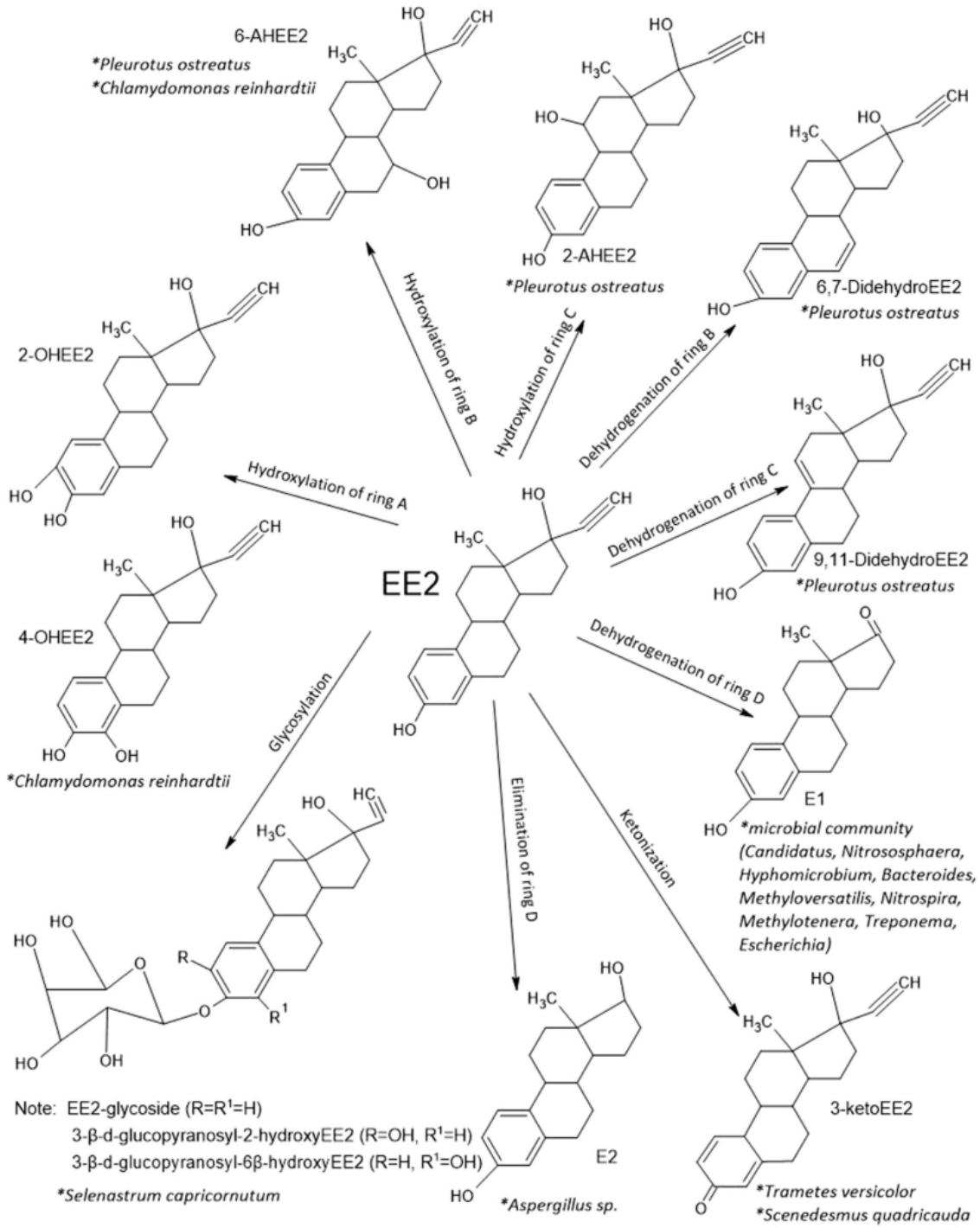


Fig. 4 Proposed biotransformation pathway of EE2. It is noted that the mark (*) is the example of a biodegrading organism

R^1 = H), and 3-β-d-glucopyranosyl-6β-hydroxyEE2 (R = H, R^1 = OH).

4.5 DES

The DES transformation by *Pseudomonas sp.* strain was obtained by Zhang et al. (2013) as seen in Fig. 5. The study detected DES-4-semiquinone formation as the first step of the DES degradation pathway. DES-4-semiquinone was transformed via dehydrogenation. A study by Liu et al. (2018b) accepted that hydroxylation, glycosylation, and methylation are the most general transformation processes or organic pollutants facilitated by microalgae.

5 Future Suggestions

It is readily reviewed that the degradation of more than 80% was achieved by some bacteria, such as BH2-1 (Pratush et al., 2020), SJTR1 strain (Xiong et al., 2018), and SJTE1 strain (Wang et al., 2019a). However, some other bacteria could only remove estrogens with efficacy below 65%, such as anaerobic strains of iron-reducing bacteria (Ivanov et al., 2010), *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Rhodococcus equi*, *Rhodococcus erythropolis*, and *Rhodococcus zopfii* (Larcher & Yargeau, 2013a). These studies had yet identified the type of bacteria most suitable for the high removal ability. Bacteria proved capable of being functional under several conditions, such as aerobic-anaerobic conditions (Li et al., 2012a) and saline, heavy metal, or surfactants-contained conditions (Xiong et al., 2020b). However, it has not been determined which types of bacteria are truly capable of being under anaerobic, aerobic, saline, heavy metal, or surfactants-contained conditions. Future studies are expected in identifying bacteria to specifically achieve 100% estrogen removal under these conditions. Furthermore, it is also unknown what types of bacteria are performing at low- and high-temperature conditions.

Most studies used several types of fungi from the Sordaricales (Lloret et al., 2010, 2012), Hypocreales (Wu et al., 2018), Polyporales (Golveia et al., 2018), and Agaricales (Křesinová et al., 2018) orders. Fungi of other orders have not been widely studied. Therefore, further research is needed to confirm the use of fungi from other orders, for example, the *Saccharomycetales* or *Auriculariales* order. Types of green algae have been widely researched to remediate pollutants, for example, algae *Scenedesmus obliquus* (Ruksrithong & Phattarapattamawong, 2019), *Haematococcus pluvialis*, *Chlorella vulgaris* (Wang et al., 2019b), *Selenastrum capricornutum* (Wang et al., 2017), *Desmodesmus subspicatus* (Maes et al., 2014), and *Raphidocelis subcapitata* (Liu et al., 2018b). Meanwhile, other algae such as red and blue algae have not been well developed to remediate estrogens. The widespread use of green algae could not diminish the estrogen compound completely, even though they have an efficiency percentage of > 90% (Wang et al., 2017). Future study is required to determine the biodegradation rate for a full degradation of estrogens so that it can be applied to WWTP efficiently.

Although the use of bacteria, fungi, and algae has been investigated and presented in this paper, exploration of other organisms such as cyanobacteria has also been prospective. Cyanobacteria have been

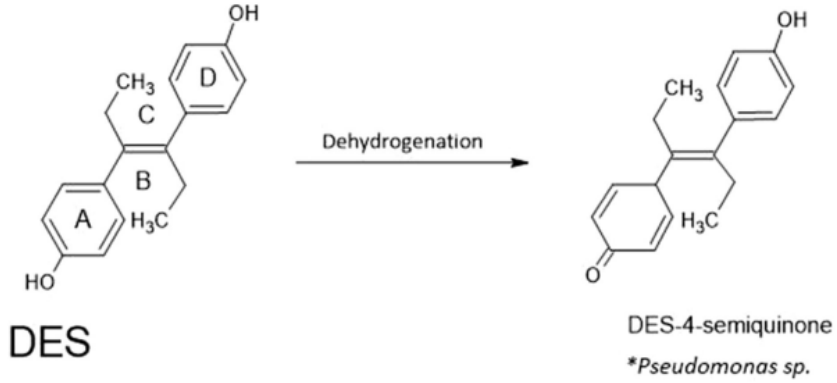


Fig. 5 Proposed biotransformation pathway of DES. It is noted that the mark (*) is the example of a biodegrading organism

well known as species responsible for harmful algal blooms in eutrophic waters but they are sometimes neglected to be used for the remediation of environmental pollutions because of the production of toxic microcystins as by-products of metabolism (Hitzfeld et al., 2000). It is potential to use cyanobacteria as a remediating agent if the release of unwanted toxins could be controlled during the process since they can grow rapidly, have high nutrient uptake capability, and need low requirement for favorable environments such as temperature and pH (Rzymski et al., 2014). Several studies have been initiated to investigate the capability of cyanobacteria for the degradation of estrogens (Bai et al., 2019; Sami & Fatma, 2019). For instance, biodegradation of E2 by using cyanobacterium *Microcystis aeruginosa* was investigated (Bai et al., 2019). At E2 concentrations of 10 μg/L and 100 μg/L, the removal efficiencies can achieve 81 ± 2% and 55 ± 3%, respectively. In general, increase in the nitrogen sources can improve the removal efficiencies while the biodegradation capacity of the proposed cyanobacterium decreased with the increase in the pollutant concentrations. Another study evaluated cyanobacterium *Spirulina* CPCC-695 for the degradation of E1 (Sami & Fatma, 2019). The study found that the biodegradation efficiency ranged from 53.7 to 94.5%. The above studies showed that estrogens pollutant can be bioremediated by using cyanobacteria and it can be a new era in the field of biodegradation of environmental pollutions.

The use of fungal residues for biodiesel production provides an alternative fuel (Vasiliadou et al., 2016). This biodiesel production has environmentally friendly properties. The residue generated from the use of biodiesel may also be friendly to the environment. Therefore, the production of biodiesel from fungal residue needs further investigation. Besides the use of fungal residues for biodiesel production, the result of degradation products also needs to be investigated. Supposing that E2 was step-polymerized to produce various oligomers (Sun et al., 2020b), the transformation of estrogens into various oligomers can reduce the use of chemicals to produce various oligomers. Therefore, the production of various oligomers is more environmentally friendly. The potential algae that can stimulate the generation of biofuel feedstock need further in-depth investigation (Ji et al., 2014). Their potential to become alternative energy to meet the world's energy needs should be researched. In addition, biofuel production can increase energy security in the environment because it is environmentally friendly. Moreover, it can reduce the long-standing global dependence on petroleum.

Kinetic studies for bacteria, fungi, and algae need to be done more deeply. In general, studies were thus far carried out using the Pseudo-first-order model. Several other models show their potential to be used. Kinetic studies using the Pseudo-first-order model had shown a good R^2 value indeed, but these studies did not state the error values. In the future, the use of error values to judging the performance of a model is highly recommended rather than the only R^2.

Almost all estrogens are transformed into estrone, such as E2 (Ye et al., 2017), E3 (Ye et al., 2019), and EE2 (Ma et al., 2018). The formed estrone takes more time to degrade. Besides taking time, the presence of estrone has a negative impact on the environment. E1 influenced hatchability, time to hatching, growth, and deteriorated development of *Oryzias latipes* (Lei et al., 2013). Hence, future studies are needed to explore other microorganisms that may cover different transformation mechanisms.

6 Conclusion

This paper reviewed critically the removal opportunities and challenges for bioremediation of natural and synthetic estrogens using microorganisms like bacteria, fungi, and algae. This paper showed that bacteria, fungi, and algae can remove estrogens from water environments with different efficacies depending on the environmental condition, type of organism, and type of estrogen. Several advantages and disadvantages of each microorganism had then been highlighted. The mathematically defined characteristics of the kinetic behaviors during the removal of estrogens from literature had also been discussed. In addition, the transformation of estrogen using these organisms showed that estrogen can be generally transformed into less toxic chemicals. Finally, the paper suggested more potential studies focusing on the evaluation of other available microorganisms that can survive under other hostile environmental conditions.

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