Environmental Pollution 157 (2009) 3325–3335

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Review

Microbial transformation of synthetic estrogen 17α-ethinylestradiol

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Current knowledge of 17α-ethinylestradiol microbial transformation is summarized.

ARTICLE INFO

Article history:
Received 1 March 2009
Received in revised form
10 June 2009
Accepted 16 June 2009

Keywords:
17α-Ethinylestradiol
Microbial biodegradation
Ligninolytic fungi
Endocrine disrupters

ABSTRACT

Natural estrogens such as estrone, 17β-estradiol, estriol, and the particularly recalcitrant synthetic estrogen 17α-ethinylestradiol used as oral contraceptive, accumulate in the environment and may give rise to health problems. The processes participating in their removal from soil, wastewater, water-sediments, groundwater-aquifer material, and wastewater or sewage treatment plant effluents may involve the action of bacterial and microbial consortia, and in some cases fungi and algae. This review discusses the different efficiencies of bacterial degradation of 17α-ethinylestradiol under aerobic and anaerobic conditions, the role of sulfate-, nitrate-, and iron-reducing conditions in anaerobic degradation, and the role of sorption. The participation of autotrophic ammonia oxidizing bacteria and heterotrophic bacteria in cometabolic degradation of estrogens, the estrogen-degrading action of ligninolytic fungi and their extracellular enzymes (lignin peroxidase, manganese-dependent peroxidase, versatile peroxidase, laccase), and of algae are discussed in detail.

1. Introduction

Synthetic and natural estrogens are a group of environmental pollutants known for their negative influence, particularly on aquatic organisms. Due to the widespread presence in the environment and endocrine activity even at low concentrations e.g. as low as 0.1 ng L^{-1} of 17α-ethinylestradiol (EE2) that induces vitellogenesis in male rainbow trout (Purdom et al., 1994; Aerni et al., 2004), these endocrine disrupters have received increased attention in water quality management and health care (Colborn et al., 1993). Many studies demonstrated that certain chemicals called endocrine disrupting compounds (EDCs) are able to mimic hormones or interfere with the action of endogenous hormones. The natural estrogens estrone (E1), 17β-estradiol (E2), estriol (E3), and particularly the synthetic estrogen EE2; which is hugely used as an oral contraceptive, bind to the estrogen receptor and can misregulate or interfere with normal biological responses. By mimicking natural hormones or disrupting signal pathways as endocrine disrupters, estrogens can stimulate the growth of human breast cancer cells (Soto et al., 1991), or induce the expression of vitellogenin in fish (Kwak et al., 2001); both mechanisms are used to prove estrogen activity in bioassays. Non-metabolized EE2 and its conjugates are excreted into wastewater. During sewage treatment, EE2 is released from the corresponding conjugates by hydrolysis and reaches the environment via the effluents of wastewater treatment plants (Tyler and Routledge, 1998). Due to incomplete removal during the waste treatment process, synthetic and natural estrogens are considered to be major contributors to the estrogenic activity associated with wastewater treatment plant effluents (Gutendorf and Westendorf, 2001; Pauwels et al., 2008a, reviewed in Clouzot et al., 2008).

This increasing environmental and public health risk requires the development of novel approaches to eliminate these compounds from the environment. However, due to certain persistency, particularly of EE2 towards microbial transformation, it is necessary to carefully identify microorganisms that are able to transform this compound and to clarify metabolic processes that can lead to the loss of estrogenic activity.

The aim of this article is to summarize current knowledge about microbial transformation of mainly EE2 by bacteria, fungi, algae and consortia of microbes during wastewater treatment processes that have been studied from this aspect.

2. Bacterial transformation

Table 1 reports the summary of degradation processes of EE2 and other EDCs; the type of treatment, the matrix in which the treatment was performed, and the removal rates are specified.

List of abbreviations: EE2, 17α-ethinylestradiol; EDCs, endocrine disrupting compounds; E1, estrone; E2, 17β-estradiol; E3, estriol; AOB, ammonia oxidizing bacteria; LiP, lignin peroxidase; MnP, manganese-dependent peroxidase; VP, versatile peroxidase; Lac, laccase; HRP, horseradish peroxidase; PPCPs, pharmaceuticals and personal care products; WWTPs, wastewater treatment plants; SRT, sludge retention time; MBR, membrane bioreactor.

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doi:10.1016/j.envpol.2009.06.027

Table 1
Transformation of EE2 and other EDCs with various treatments.

Type of degradation	EDCs studied	EE2 degradation	Degradation conditions	Notes	References
Cultures established from lake water and sediments	EE2, and E2	no anaerobic degradation of EE2 (initial concentration 5 mg L^{-1}) over long incubation period (over three years)	methanogenic, sulphate-, iron-, and nitrate-reducing anaerobic conditions	E2 transformed to estrone	Czajka and Londry, 2006
Water-sediments, groundwater-aquifer material	EE2, E2, bisphenol A, and 4-n-nonylphenol	rapid degradation of all EDCs in both media with >90% of them within the first 2–4 d under both conditions	aerobic conditions and sulfate-, nitrate-, and iron-reducing anaerobic conditions	90% dissipation time under anaerobic conditions >1000 d (EE2)	Sarmah and Northcott, 2008
Water-sediments, groundwater-aquifer material	EE2, E2, bisphenol A, 4-tert-octyl phenol, 4-n-nonylphenol	no degradation of EDCs under anaerobic condition, under aerobic condition EE2 concentration decreased within 70 d from 1 to 0.62 μg g^{-1} in the aquifer material	aerobic and anaerobic conditions, sorption test at room temperature	highest sorption to the aquifer material had 4-*n*-Nph, E2 and EE2 had modest sorption	Ying et al., 2003
Groundwater-aquifer material	E2, EE2, bisphenol A, 4-t-octylphenol and 4-n-nonylphenol	under aerobic conditions rapid degradation of EDCs except EE2 (initial conc. 5 mg L^{-1}), the EE2 half-lifes about 26d in groundwater and 15d in the effluent microcosm	microcosm with aquifer material and groundwater mixture or effluent mixture in the presence of glucose under both aerobic and anoxic conditions	under anoxic conditions biodegradation only of E2 in either water type	Ying et al., 2008a
Nitrosomonas europaea from nitrifying activated sludge	E1, E2, E3, and EE2	first order reaction kinetics 0,035h^{-1} for EE2, 0.030 h^{-1} for E3, 0.056 h^{-1} for E1, and 1.3 h^{-1} for E2 (initial conc. 0.4 mg L^{-1})	batch experiment using nitrifying activated sludge from Tokio and using isolated strain *N. europaea*	addition of allylthiourea reduced the estrogen-degrading activity	Shi et al., 2004
Nitrosomonas europaea and *Nitrosospira multiformis* from nitrifying activated sludge	EE2	EE2 (initial concentration 300 mg L^{-1}) removal via nitration	batch tests with NH4-N addition (200–500 mg L^{-1})	nitrated degradation products, at high NH4-N concentration degradation by AOB, or to abiotic nitration, at low concentration due to heterothropic bacteria	Gaulke et al., 2008
Ammonium monooxygenase containing culture extract	EE2	biotransformation via EE2-hydroxylation on an A ring	nitrifying bioreactor with sludge recycle operated at HRT of 0.75d and SRT of 20d	relationship between nitrification and EE2 removal in enriched nitrifying cultures	Yi and Harper, 2007
Nitrifying activated sludge	E1, E2, and EE2	high biological stability of EE2 compared to with E1	activated sludge from sequencing batch reactor (stored max. three weeks at 5–8 °C)	involvement of ammonia monooxygenase in biotransformation of EE2 into EE2-OH	Ren et al., 2007b
Nitrifying activated sludge	EE2	biodegradation of EE2 more important than biosorption under the condition of the high initial ammonia concentration (>48 mg L^{-1})	sorption and biodegradation in laboratory scale bioreactors	relationship between biomass particle size, hydrophobicity, and sorption capacity	Yi et al., 2006
Acinetobacter, *Agromyces*, and *Sphingomonas*	E1, E2, E3, and EE2	under aerobic conditions, degradation rates of estrogens increased with the initial concentration (range 50–200 μg L^{-1})	aerobic and anoxic conditions in microcosm constructed with marine sand and ultra filtered secondary effluent	EE2 remained stable during cultivation with all the three isolates	Ke et al., 2007
Rhodococcus zopfii and *R. equi*	E1, E2, E3 and EE2	*R. zopfii* removed completely 100 mg L^{-1} of EE2 within 24 h	cultivation under aerobic conditions in test tubes at 25 °C for 24 h	enrichment culture of activated sludge from Japanese STWs	Yoshimoto et al., 2004
Sphingobacterium sp. JCR5	E1, E2, E3, EE2 and mestranol	removal of 87% of the substrate added (30 mg L^{-1}) within 10 days	bacterial strain isolated from STW of an oral contraceptives producing factory in China	metabolic pathway for EE2 degradation	Ren et al., 2007a
Laccase from *Trametes* sp. and *Pycnoporus coccineus*	EE2 adsorbed on sea sand	removed EE2 within 48 h in the test tube to the extent of 90%	in a test tube and a rotating reactor	laccase activity of 0.8 U mL^{-1}	Tanaka et al., 2001
Manganese peroxidase and the laccase from *P. chrysosporium* and from *T. versicolor* cultures	E2, and EE2	E2 and EE were completely transformed within 1 h, estrogenic activity disappeared within 1h also	mediator system with 1-hydroxybenzotriazole	MnP activity of 10 nkat mL^{-1}, monitored also the course of estrogenic activities	Suzuki et al., 2003
Horseradish peroxidase	E1, E2, E3, and EE2	at pH 8 removed 96–100% of E2, E3, and EE2 within 1 h	effect of temperature and pH on enzymatic kinetics	HRP activity of 0.017 U mL^{-1}	Auriol et al., 2006
Laccase	Steroid estrogens	laccase was able to achieve complete removal	synthetic water and municipal wastewater	laccase (20 U mL^{-1}), 1-hydroxy-benzotriazole - mediator, improved laccase-catalyzed system efficiency	Auriol et al., 2007a
Trametes versicolor laccase and HRP	E1, E2, E3, and EE2	estrogens (100 ng L^{-1}) were completely oxidized with both the enzymes in the wastewater reaction mixtures after a 1h treatment	batch reaction at 25 °C with the buffered (pH 7) reaction mixture or wastewater	laccase activity of 20 U mL^{-1} HRP activity of 8–10 U mL^{-1}	Auriol et al., 2008

Table 1 (*continued*)

Type of degradation	EDCs studied	EE2 degradation	Degradation conditions	Notes	References
T. versicolor	EE2, and E2	EE2 removal in the batch 0.44 mg L^{-1} h^{-1} (initial conc. 7.3 mg L^{-1}) in the bioreactor 0.09 mg L^{-1} h^{-1} (initial conc. 10 mg L^{-1})	experiments in the batch, and the bioreactor	the technical feasibility of fungal treatment (estrogens) using continuous bioreactor with suspended fungal biomass	Blánquez and Guieysse, 2008
Irpex lacteus, *Bjerkandera adusta*, *Phanerochaete chrysosporium*, *Phanerochaete magnoliae*, *Pleurotus ostreatus 3004*, *Trametes versicolor*, *Pycnoporus cinnabarinus*, *Dichomitus squalens*	EE2, bisphenol A, irgasan, 4-n-nonylphenol, and 4-nonylphenol	*I. lacteus* and *P. ostreatus* totally degraded EE2 within 3 days (initial concentration 10 mg L^{-1})	static cultures incubated with EDCs and harvested after 3, 7 and 14 days	determination of estrogenic activity using a recombinant yeast assay	Cajthaml et al., 2009
Cephalosporium aphidicola *Cunninghamella elegans*	EE2, and norethisterone	EE2 initial concentration 0.2 mg mL^{-1} media	experiment in a liquid medium	work focused on identifying metabolites of EE2 and norethisterone	Choudhary et al., 2004
pathogenic ascomycete *Fusarium proliferatum* strain HNS-1	EE2	97% of the EE2 removed from the culture after 15 days (initial concentration 25 mg L^{-1})	batch experiment	isolating EE2 degrading microorganism	Shi et al., 2002
17 different natural soils	E1, E2, and EE2 (unlabeled and ^{14}C-labelled)	E2 and E1 degraded with much greater degree than EE2 (initial concentrations 120, 60, 12, 6 and 1.2 μg g^{-1}) in all tested soils	laboratory microcosm incubations with addition of ammonium nitrate, alanine, glucose, catechol or streptomycin	two grassland soils, two forest and 14 agricultural soils	Stumpe and Marschner, 2009
Agricultural land	E1, E2, and EE2 (E1, E2 were ^{14}C labelled compounds)	E2 and EE2 were rapidly removed in soil conditions typical of a temperate growing season (initial concentrations from 0.1 to 10 mg kg^{-1})	three soils varying widely in texture and properties, a sandy loam, and silt loam were used in laboratory microcosm incubations	EE2 stability was evaluated in the absence of oxygen	Colucci and Topp, 2001a,b
Agricultural land	E1, E2, EE2, and 4-nonylphenol	half-lives ranging from a few hours to a few days	soil microcosms in laboratory incubation with the C^{14} or H^3 labelled compounds	estrogens were rapidly removed from aerated soils under temperate growing conditions	Lorenzen et al., 2006
Four various soils	E1, E2, E3, EE2 and other EDs	under aerobic conditions degradation within 7 d, under anaerobic no degradation of the chemicals except for E2	sorption test using a batch equilibrium method, biodegradation experiments under aerobic and anaerobic conditions	estrogens were adsorbed on soils in the order EE2, E2, E1, E3	Ying and Kookana, 2003
WWTP and activated sludge	E1, E2, E3, EE2, and nonylphenol	the highest estrogen removal in the STW which only used primary treatment	estrogens in the effluent of 17 STW included primary chemical treatment only, submerged aerated filter, oxidation ditch, activated sludge, and trickling filter combined with activated sludge	EE2 was detected only in effluents of two STWs	Johnson et al., 2005
WWTPs	E1, E2, E3, and EE2	E1 and EE2 were more persistent during the treatment than the other estrogens	four South Australian STWs with different technologies	the least efficient STWs was consisted of a series of anaerobic and aerobic lagoons	Ying et al., 2008b
WWTP	E1, E2, E3, EE2, and nonylphenols	EE2 removal only 3% (24 h), during the 7 day treatment period removal increased only up to 5.6%.	nitrifying activated sludge plant in England	excellent removal for other estrogens and nonylphenols (97–99%) and 98% removal of estrogenic activity	Kanda and Churchley, 2008
WWTP and receiving water	E1, E2, and EE2	higher estrogens removal linked to higher biomass concentration	relationship between the equivalent biomass concentration and estrogens degradation rate constant in various environments	The EE2 correlation $R2 = 0.73$ between the logarithm of the rate constant and the corresponding logarithm equivalent biomass concentration	Cao et al., 2008
WWTP	E1, E2, E3, EE2 and their conjugates form	>90% removal estrogen concentration and estrogenicity during biological treatment, E1, E2 and EE2 persisted in the treat water below 10 ng L^{-1}	estrogens inlet concentration 200–500 ng L^{-1}, estrogenic activity between 25 and 130 ng L^{-1} of E2 equivalents	small fraction sorbed to the sludge, the main vehicle of estrogen elimination was biodegradation	Muller et al., 2008
Activated and inactivated sludge	biphenol A, E2, and EE2	high adsorption affinity of the compounds to the adsorbent (initial concentration 2.5 0×10^{-5} to 50 mg L^{-1})	inactivation of the sludge with 0.5 ml L^{-1} mercury(II)sulphate	the adsorption was found to depend on pH	Clara et al., 2004
Municipal WWTPs	E1, and EE2	the efficiency (>90%) of E1 and EE2 removal	various redox conditions in batch experiment	the importance of aerobic conditions for the removal of all estrogens	Joss et al., 2004

(*continued on next page*)

Table 1 (*continued*)

Type of degradation	EDCs studied	EE2 degradation	Degradation conditions	Notes	References
Municipal WWTP and industrial WWTP	^{14}C-labelled estrogenic compounds	the mineralization of EE2 in MTP during 24 h, with only 40% mineralized to $^{14}CO_2$ (25–75 fold less than that of E2)	biosolids from four municipal treatment plants and one industrial plant	the mineralization of estrogens in MTP 70–80% during the 24 h, in ITP only 4%	Layton et al., 2000
Activated sludge	E1, E2, and EE2	no significant loss of the sum of estrogens	under strictly anaerobic conditions	adsorption to sludge accounted for a 32–35% loss from the liquid phase	Mes et al., 2008
Activated sludge biomass	EE2	spontaneous sorption EE2 to activated sludge (initial concentration 100–500 μg L^{-1}) primary due to physisorption and low-level chemisorption	experiments with biomass from membrane bioreactor and sequencing batch over a range of temperatures in short time frame (0–25 min)	to prevent biodegradation sodium azide addition	Xu et al., 2008
Activated sludge	EE2	under aerobic conditions 22% removal, under anoxic conditions EE2 persistent	relationship between availability of oxygen, nitrification rate, and estrogen removal	the higher removal of estrogens associated with higher nitrification rate	Dytczak et al., 2008
Nitrite-accumulating sequencing batch reactors	E1, E2, and EE2	EE2 removal adversely affected with SRT shorter than 5.7 days and significantly lower when SRT longer than 7.5 days	two nitrite-accumulating sequencing batch reactors with different sludge ages operating under aerobic conditions and anoxic/anaerobic/aerobic conditions	cause of nitrite toxicity and the inhibition of the monooxygenase (AMO) and the microbial population	Pholchan et al., 2008
Aerated nitrifying submerged fixed bed bioreactors	E2, and EE2	complete EE2 removal from the synthetic STWs at loading rates 11 μg L^{-1} d^{-1} (initial concentration from 11 to 143), 90% removal of EE2 (80 μg L^{-1}) in reactor within 12 days	lab-scale reactors (volume 1.4 l) with a flow velocity 1 m h^{-1} and continues aeration and the batch reactors	in the batch test, no or little removal of EE2 in heat-treated effluent, EE2 removal by AOB continued also after several months of starvation	Forrez et al., 2009a
Membrane bioreactor	two differently labelled radioactive forms of EE2	radioactivity mainly remained sorbed in the reactor (removal of 80%), the real mineralization <1%, EE2 conc. 8 g L^{-1}	a membrane bioreactor (MBR) operating with 25 days SRT during the whole experimental period (35 days)	elimination pathway does not involve the removal of the ethinyl group from EE2 molecule	Cirja et al., 2007
11 microalgae strains	EE2	*Selenastrum capricornutum*, *Scenedesmus quadricauda*, *S. vacuolatus* and *Ankistrodesmus braunii* were able to biotransform the substrate	10 mg of EE2 to 50 mL of axenic algal cultures with an initial concentration of 160 mg L^{-1} dry weight	identified several transformation products	Della Greca et al., 2008

To assess the role of bacterial degradation in the fate of estrogenic steroids in the environment, the transformation of estrogens was studied under both aerobic and anaerobic conditions. Czajka and Londry (2006) investigated the potential for anaerobic biodegradation of EE2 and E2 using cultures established from lake water and sediments. No anaerobic degradation of EE2 (5 mg L^{-1}) was observed in their experiments. Although E2 could be transformed to estrone, the complete degradation of estrogens was minimal, suggesting that they accumulate in anoxic environments. On the contrary, a rapid degradation of four estrogens, including EE2, was observed in river water-sediments and groundwater-aquifer material under aerobic conditions (Sarmah and Northcott, 2008). The 90% dissipation time values for all compounds ranged from 0.9 to 2.8 days under both conditions. Under anaerobic conditions, however, the 90% dissipation time values for EE2 exceeded 1000 days. Anaerobic degradation of estrogenic compounds in this study was attributed to the sulfate-, nitrate-, and iron-reducing conditions. It was also postulated that overall degradation of the compounds was influenced by abiotic factors accounting for up to 40% degradation. The authors suggested that these abiotic factors could include hydrolysis, chemical reduction, photolysis, irreversible sorption or volatilization. Furthermore, they stated that E2, EE2 can also undergo surfaced-induced abiotic transformation due to the catalytic effect of various clay minerals.

Similarly, Ying et al. (2003) reported an aerobic degradation of E2 and 4-n-nonylphenol in the aquifer material with a half-life of the chemicals equal to 2 and 7 days. EE2 was degraded slowly with an estimated half-life of 81 days, with little or no degradation of estrogens observed within 70 days under anaerobic conditions in native groundwater. In another experiment Ying et al. (2008a) described faster degradation of EE2 under aerobic conditions in effluent supplemented or unsupplemented aquifer material. EE2 had a half-life of 15 days compared with 26 days in the aquifer material and groundwater microcosm. Biodegradation was not significant under anoxic conditions. Higher resistance of EE2 to biodegradation, when compared to E2, was proven also with microorganisms from water samples of English and Japanese rivers (Jurgens et al., 2002; Matsuoka et al., 2005). Aerobic degradation of five endocrine disrupting compounds – bisphenol A, E2, EE2, 4-tert-octyl phenol, and 4-n-nonylphenol – in seawater collected from the coastal area of South Australia demonstrated that the chemicals could be degraded by aerobes in seawater once the microbes in water became acclimated to the chemicals (Ying and Kookana, 2003). The study showed that EE2 (together with bisphenol A and E2) was degraded in water within 56 days with a lag phase preceding its fastest degradation. EE2 was also found to be degraded in the marine sediment under aerobic conditions ($t1/2 > 20$ d) while under anaerobic conditions no degradation was noted.

In comparison with aquifer and marine environments, relatively fast EE2 degradation was reported in agricultural soils and nitrifying activated sludge (Vader et al., 2000; Colucci and Topp, 2001a,b). Shi et al. (2004) demonstrated the link between the ammonia oxidizing bacteria (AOB) from nitrifying activated sludge and biodegradability of natural and synthetic estrogens, including EE2. The addition of allylthiourea, an ammonia oxidation inhibitor, significantly reduced the estrogen-degrading activity of activated sludge and inhibited the growth of AOB. These results suggested that other microorganisms apart from AOB are also involved in estrogen degradation by nitrifying activated sludge. The estrogen

degradation potential of AOB was further demonstrated with an isolated strain of *Nitrosomonas europaea* when the strain was able to decompose 1 mg L^{-1} of EE2 within 96 h during a batch experiment. Its ability of estrogen degradation was also inhibited by allylthiourea (Shi et al., 2004). Later tests in a stirred tank bioreactor showed a linear relationship between nitrification and EE2 removal by enriched cultures of autotrophic ammonia-oxidizers; this implied cometabolic transformation of EE2 by the organisms (Yi and Harper, 2007). The cometabolism of AOB dominated also the degradation of estrone and estradiol in nitrifying activated sludge (Ren et al., 2007b). The appearance of EE2-OH as a metabolite then provided evidence for an involvement of ammonia monooxygenase in AOB EE2 transformation. The role of the enzyme in the process was proven under in vitro conditions when EE2 was degraded in the presence of an ammonium monooxygenase-containing culture extract (Yi and Harper, 2007). Gaulke et al. (2008) isolated two ammonia oxidizing bacterial strains, *N. europaea* and *Nitrosospira multiformis*, and described EE2 removal under NH_4 supplemented conditions. On the basis of their results they suggested that EE2 removal at low nitrogen (NO_2) concentration, usually found in municipal treatment activated sludge systems, is most likely due to organotrophic bacteria rather than the ammonia oxidizing bacteria. In experiments testing EE2 sorption to nitrifying activated sludge, biosorption of EE2 by the biomass was shown to be affected by the initial ammonia concentration (Yi et al., 2006). At higher initial ammonia concentrations the role of biodegradation became more important in EE2 removal than its biosorption.

Recently, several studies characterized the estrogen degradation potential of various isolated bacterial strains. The first E2 degrading bacterium *Novosphingobium tardaugens* was isolated from activated sludge (Fujii et al., 2002). However, not all estrogen-degrading bacteria were shown to degrade recalcitrant EE2. Ke et al. (2007) isolated three strains that belonged to genera *Acinetobacter*, *Agromyces*, and *Sphingomonas*, and were able to degrade E1, E2, and E3 under both aerobic and anoxic conditions. Nevertheless, EE2 remained stable during cultivation with all the three isolates. On the other hand, *Rhodococcus zopfii* and *Rhodococcus equi* isolated from activated sludge of Japanese wastewater treatment plants were shown to degrade all the four principal estrogens (E1, E2, E3 and EE2) (Yoshimoto et al., 2004). *R. zopfii* Y50158 showing the strongest degradation activities removed completely 100 mg L^{-1} of EE2 within 24 h in this study. Another EE2-degrading bacterium, isolated from activated sludge of a wastewater treatment plant in China, was identified as *Sphingobacterium* sp. JCR5 (Ren et al., 2007a). It grew on EE2 as the sole source of carbon and energy and metabolized up to 87% of the substrate added (30 mg L^{-1}) within 10 d. In addition to EE2, the strain could be cultivated on other estrogens such as E1, E2, E3, and mestranol. Analysis of EE2 degradation showed that in the first step it was oxidized to E1 with a subsequent ring cleavage to 2-hydroxy-2,4-dienevaleric acid and 2-hydroxy-2,4-diene-1,6-dioic acid, which were the main catabolic intermediates (Ren et al., 2007a). The metabolic pathway for EE2 degradation by *Sphingobacterium* sp. JCR5 suggested in that work shared a certain analogy with previously reported testosterone degradation by *Comamonas testosteroni* (Horinouchi et al., 2003). In the latest study, six strains of *Proteobacteria* were added to the list of strains capable of EE2 transformation (Pauwels et al., 2008b). Bacterial strains isolated from compost cometabolized EE2 at low $\mu g L^{-1}$ levels when metabolizing E1, E2, or E3. No other metabolites beside E1, E2, E3 and EE2 were detected, suggesting that total degradation and fission of aromatic rings occurred.

Generally it can be summarized that bacterial EE2 transformation is significantly effected by oxygen with higher degradation efficiency under aerobic conditions. Relatively fast bacterial degradation was recorded in agriculture soils and nitrifying activated sludge. In this case, the key role in transformation process could be attributed to AOB showing highest removal at high initial ammonia concentrations. Even higher degradation efficiencies (tens mg L^{-1} of EE2) were recorded with *Rhodococcus* and *Sphingobacterium* sp. isolated from activated sludges.

3. Ligninolytic fungi, ligninolytic enzymes and other peroxidases

Ligninolytic fungi produce extracellular enzymes with low substrate specificity, which makes them suitable for degradation of various aromatic compounds, even those with low water solubility. The ligninolytic system consists of four major enzymes: lignin peroxidase (LiP, E.C. 1.11.1.14), manganese-dependent peroxidase (MnP, E.C. 1.11.1.13), versatile peroxidase (VP, E.C. 1.11.1.16) and laccase (Lac, E.C. 1.10.3.2). The physiology of the production of the enzymes together with their application for transformation of estrogenic compounds has been studied in the recent years.

In an early study from 1973, Lugaro et al. (1973) demonstrated the ability of laccase from *Polyporus versicolor* in a biphasic system to oxidise E1, E2, and E3. These authors detected several transformation products of E1 and E3 but their structures were not elucidated. Laccases from *Trametes* sp. and *Pycnoporus coccineus* (0.8 U mL^{-1}) were tested for transformation of EE2 (2 mol kg^{-1} of sand) adsorbed on sea sand in a test tube and a rotating reactor (Tanaka et al., 2001). The enzyme from *Trametes* sp. and *P. coccineus* removed EE2 within 48 h in the test tube to the extent of 90% and 80% respectively. The laccase from *Trametes* sp. was then tested in the rotating reactor, reaching 80% degradation efficiency within 8 h. Tanaka et al. (2001) studied biodegradation of E1 and E3 (3 mol g^{-1}) with laccases purified from *P. coccineus* (0.8 U mL^{-1}) on a model sediment in a rotating reactor. EE2 and E1 were degraded in the system within 48 h to 80% and 60%, respectively.

Suzuki and co-authors (2003) investigated the removal of the steroidal hormones E2 and EE2 with manganese peroxidase (MnP) (10 nkat mL^{-1}), by the laccase-mediator system with 1-hydroxy benzotriazole prepared from *Phanerochaete chrysosporium* ME-446 and *Trametes versicolor* IFO-6482 cultures in organic solvent and under biphasic conditions. They also monitored the course of estrogenic activities. The authors found that 10^{-5} M E2 and EE2 were completely transformed within 1 h when estrogenic activity dropped to approx 10% of the original values and the activity disappeared in 8 h. This residual activity could be caused by the presence of active transformation products, however, the authors did not succeeded in detecting them using HPLC technique. Laccases from a species of the imperfect fungus *Myceliophthora* (27.5 U mL^{-1}) and from the ligninolytic strain *Trametes pubescens*, both adsorbed on glass beads, were used for the transformation of natural estrogen E2 (5 g L^{-1}) (Nicotra et al., 2004). The reaction products were tentatively identified as C–C dimeric products with coupling between either 4′- 4, or 2′- 2, and C–O (phenolic ring) at 4′or 2′ positions and the structures are shown in Fig. 1. Tamagawa et al. (2006) tested degradation of the natural hormone E1 (10^{-4} M) by the ligninolytic fungus *Phanerochaete sordida* under ligninolytic conditions in low-nitrogen and high-carbon culture medium. They recorded 95% transformation within 5 days of incubation. The authors treated E1 (10^{-5} M) also with MnP and laccase from the same fungus and found a complete disappearance of E1 after 1 h. Complete removal of the estrogenic activity of E1 after 2 h was confirmed using the yeast two-hybrid assay system. Also in this case the remaining activity (1–3%) after 1 h treatment could be caused by formation of active transformation products or by residual concentration of original compounds that were already below the detection limit of the used HPLC method.

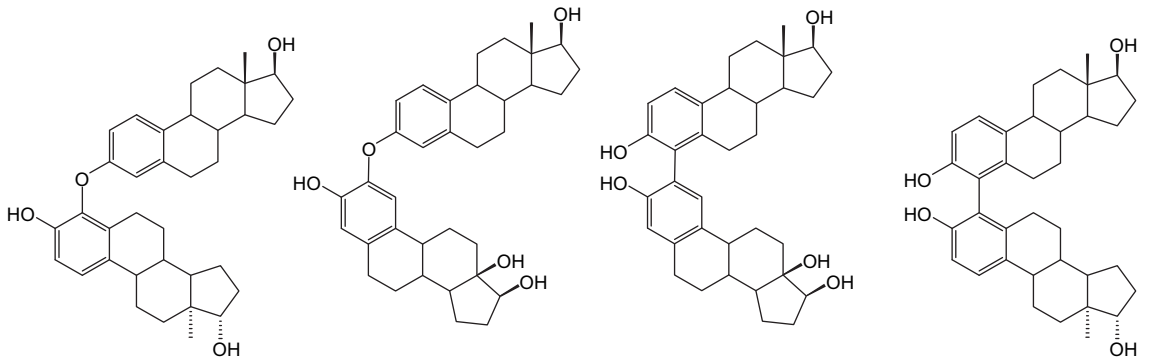

Fig. 1. Fungal laccase-mediated oxidation products of E2 in organic solvent (Nicotra et al., 2004).

Auriol and co-workers (2006) tested the feasibility of application of another peroxidase from horseradish (HRP) for biodegradation of E1, E2, E3, and EE2 (400 nM). They investigated the effect of temperature and pH on enzyme kinetics. With an HRP activity of 0.017 U mL^{-1} at pH 7 and 25 °C they found that the enzyme was able to transform 92–100% of the compounds within 1 h. They also conducted other degradation experiments in pure and filtered wastewater from activated sludge, assessing the impact of wastewater constituents on estrogen removal during enzymatic oxidation. These results showed that HRP (0.068 U mL^{-1}) at pH 8 removed 96–100% of E2, E3, and EE2 within 1 h, whereas the E1 removal was only 73%. Moreover, they demonstrated that the fastest degradation was in the case of EE2. For wastewater samples lower removals were achieved for E1, E2, E3, and EE2, specifically 29%, 19%, 28%, and 37%, respectively. The same authors performed a similar study with laccase-catalyzed conversion (*T. versicolor*) of these estrogens (Auriol et al., 2007a). The optimal pH for each studied steroid estrogen oxidation was approximately 6 in synthetic water. This research also focused on the wastewater matrix effect on developed enzymatic treatment. At pH 7.0 and 25 °C, the experiments showed that the laccase-catalyzed system for the removal of steroid estrogens was not significantly affected by the municipal wastewater matrix. The authors tested several laccase initial activities, and 20 U mL^{-1} was sufficient to achieve a complete removal of studied steroid estrogens in both synthetic water and municipal wastewater. Moreover, 1-hydroxy-benzotriazole, when used as a mediator, improved laccase-catalyzed system efficiency. The same group of authors optimized HRP enzyme-catalyzed process in a real wastewater matrix from a municipal wastewater treatment plant (Auriol et al., 2007b). The impacts of the reaction conditions, mainly including enzyme and H_2O_2 doses were investigated. The optimum molar H_2O_2/estrogens ratio in both synthetic water and actual wastewater was approximately equal to the theoretical ratio of 0.5. A kinetic study showed that the oxidative conversion of steroid estrogens catalyzed by HRP followed Michaelis–Menten kinetics in the substrate concentration range studied. The determination of K_M for each estrogen showed that HRP had an increasing affinity for E1, E3, E2, and EE2 (K_M = 7.47; 5.25; 1.44; 1.32 μM in the same order) under the experimental conditions. In real activated sludge process effluent, an HRP dose of 8–10 U mL^{-1} was required to completely remove all of the studied estrogens, while only 0.032 U mL^{-1} of HRP was necessary to treat synthetic water containing the same estrogen concentrations (from 1.1 to 17.8 ng L^{-1}). Removal of estrogenic activity and transformation with *T. versicolor* laccase (20 U mL^{-1}) and HRP (8–10 U mL^{-1}) of E1, E2, E3, and EE2 in a municipal wastewater were studied by Auriol et al. (2008). The authors found that these steroid estrogens were completely oxidized with both the enzymes in the wastewater reaction mixtures after a 1 h treatment. K_M values were determined also for laccase at pH 7 and 25 °C: E1 3.40, E2 3.99, E3 2.65, and EE2 3.78 μM. It was also demonstrated that the enzymatic treatments were very efficient in removing the estrogenic activity of the steroid estrogens using the recombinant yeast assay.

T. versicolor was also demonstrated to be able to remove EE2 in batch and continuous cultures (Blánquez and Guieysse, 2008). In batch, E2 and EE2 initially supplied at 10 mg L^{-1} were removed from more than 97% in 24 h, which corresponded to removal rates of 0.43 and 0.44 mg L^{-1} h^{-1}, respectively. A bioreactor inoculated with *T. versicolor* pellets was then continuously operated during 26 days at a hydraulic retention time of 120 h. E2 and EE2 were completely removed at removal rates of 0.16 and 0.09 mg L^{-1} h^{-1}, respectively, when fed at 18.8 and 7.3 mg L^{-1}, respectively. The authors attributed the degradation mainly to laccase activity of *T. versicolor*. Removal of several compounds with endocrine disrupting activity including EE2 in static cultures using *T. versicolor*, and other seven white rot fungi strains were described (Cajthaml et al., 2009). The most efficient degraders were found to be *Irpex lacteus*, *Pleurotus ostreatus*, and *Pycnoporus cinnabarinus*, which were able to completely remove EE2 from the initial concentration 10 mg L^{-1} within the first 3 days of incubation. Estrogenic activity generally decreased with the progress of degradation; however, in some cases during the metabolism of EE2 the results showed a residual (*I. lacteus*, *P. ostreatus* and *P. cinnabarinus*) or increased (*Phanerochaete magnoliae*) estrogenic activity, suggesting the production of metabolic intermediates with estrogenic activity.

4. Other fungi

Choudhary et al. (2004) performed a metabolic study on transformation of norethisterone by *Cephalosporium aphidicola* and subsequently of EE2 by the zygomycete *Cunninghamella elegans* in a liquid medium. The authors identified several metabolites of EE2 by *C. elegans* (Fig. 2). The transformation reactions included several hydroxylations of EE2 and in one case a subsequent methoxylation of the hydroxyl derivative. The pathogenic ascomycete *Fusarium proliferatum* was selected from sediments via a set of enrichment experiments with EE2 as the sole source of carbon (Shi et al., 2002). In a subsequent batch experiment, the fungus was proved to be able to utilize EE2 (25 mg L^{-1}) and 97% of the compound was removed from the culture after 15 days with a first-order rate constant 0.6 d^{-1}.

5. Transformation in soil

Colucci and Topp (2001a,b) evaluated the persistence and pathways of dissipation of E1, E2, and EE2 in soil by means of laboratory microcosm incubations. In the case of E1 and E2 they

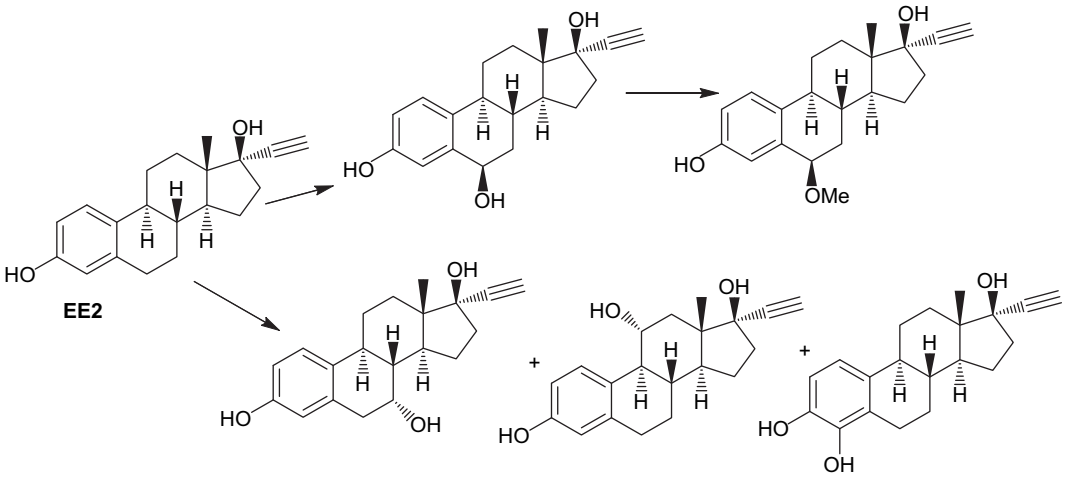

Fig. 2. Transformation products of EE2 by *C. elegans* (Choudhary et al., 2004).

used ^{14}C labelled compounds. The dissipation and mineralization rates were determined in a range of temperatures and moistures. E2 and EE2 were rapidly removed in soil conditions typical of a temperate growing season. E2 compound was oxidized to estrone in both autoclaved and nonsterile loam, silt loam, and sandy loam soils, suggesting an abiological transformation. In contrast, E1 was stable in autoclaved soil, suggesting that its removal was microbially mediated. Both E2 and E1 formed non-extractable residues, and soil-bound residues were only slowly mineralized, suggesting that their bioavailability was low. This was proved by the loss of estrogenic activity. EE2 stability was also evaluated in the absence of oxygen, and the response of dissipation kinetics to variation in temperature and moisture suggested that the removal was microbially mediated.

In a recent study from 2009, Stumpe and Marschner (2009) characterized mineralization of radiolabelled estrogens in 17 different soils under various conditions. Although sorption parameters in this study varied greatly for E2 (K_F = 21.9–317.5 mL^{-1}), for E1 (K_F = 46.0–517.5 mL^{-1}) and for EE2 (K_F = 29.9–326.1 mL^{-1}) this apparently did not control estrogen bioavailability since it showed no effects on hormone mineralization. Soil supplementation with glucose, ammonium nitrate, streptomycin etc. had a stimulating or inhibiting effect on estrogen mineralization depending on the type of the soil. On the basis of these experiments with various added substrates it is clear that white rot fungi were responsible for EE2 degradation while bacteria were responsible for E2 degradation.

A study was undertaken by Lorenzen et al. (2006) to assess the persistence of estrogenic substances in soil that could reach agricultural land via fertilization with organic amendments containing E1, E2, and EE2. The compounds rapidly dissipated in soils with half-lives ranging from a few hours to a few days. The authors concluded that the risk of these chemicals to water was low. The compounds were rapidly removed from aerated soils under temperate growing conditions; this indicates that application methods that minimize preferential flow, or runoff, of animal or human wastes should protect adjacent water from contamination.

Ying and Kookana (2003) tested sorption behaviour of several endocrine disrupting compounds including E1, E2, E3, and EE2 on four various soils. They characterized the biotransformation of E2–E1 in the loam soil under aerobic and anaerobic conditions. Sorption test using a batch equilibrium method demonstrated that estrogens were adsorbed onto soils in the order EE2, E2, E1, and E3. This laboratory study showed that the compounds were degraded rapidly in the soil within 7 days under aerobic conditions. However, during the 70-day study under anaerobic conditions in the soil, little or no degradation of the chemicals was recorded except for E2. The calculated half-lives for E2, which was found to be bio-transformed to E1 under both aerobic and anaerobic conditions, were 24 d in the soil.

6. Wastewater treatment plants and sludge degradation

Most recently, Miège et al. (2009, see references therein) summarized the data about the fate of pharmaceuticals and personal care products (PPCPs) in wastewater treatment plants (WWTPs). The authors took into account the dissolved aqueous phase without batch experiments, and created a database in order to quantitatively assess the occurrence and removal efficiency of PPCPs including natural hormones and EE2 in WWTPs. Various WWTP processes were evaluated and it was found that EE2 was highly removed (70%) in the dissolved aqueous phase by a fixed biomass reactor with high sludge retention time and waste stabilisation ponds. The processes using activated sludge with nitrogen treatment were substantially more efficient than treatments. They also found a tendency between higher EE2 influent concentrations and removal extents.

Earlier, Johnson et al. (2005) compared 17 WWTPs in Europe based on the concentrations of nonylphenol and steroid estrogens in effluents. Treatment processes included primary chemical treatment only, submerged aerated filter, oxidation ditch, activated sludge, and trickling filter combined with activated sludge. The lowest estrogen removal was observed in the WWTP which only used primary treatment. In this study EE2 was detected only in effluents of two WWTPs.

Similarly, Ying et al. (2008b) described the degradation of EE2 and other estrogens in four South Australian WWTPs with different technologies. The removal rates for the estrogens were variable but consistent with the plant performance parameters such as biochemical oxygen demand, suspended solids and ammonia, and the least efficient WWTP was that which consisted of a series of anaerobic and aerobic lagoons. The study showed that E1 and EE2 were more persistent during the treatment than the other estrogens. A poor EE2 removal was also detected in a WWTP with nitrifying activated sludge system situated in England (Kanda and Churchley, 2008). The results of a 24-h treatment experiment showed excellent removal for other estrogens and nonylphenols (97–99%), and a 98% removal of estrogenicity. However, the

removal of EE2 was only 3% which during the 7-day treatment period only increased to a mere 5.6%.

Braga et al. (2005a) reported a poor removal in an enhanced primary WWTP compared to an advanced WWTP in Australia. The difference between those two plants was primarily linked to plant performance, but the extent to which the removal of steroid estrogens was due to bacterial metabolism rather than adsorption to the bacterial biomass remained unclear. EE2 was not detected during the study period in the influent or effluent of either WWTP. Concentrations at ng g^{-1} levels of EE2 were detected in the waste activated sludge of the two WWTPs (Braga et al., 2005b), but not in the raw sewage, suggesting that EE2 is resistant to biological treatment and is primarily removed due to sorption to sludge biomass. This agreed with previously reviewed data (Johnson and Sumpter, 2001). Muller et al. (2008) surveyed E1, E2, EE2 and their conjugated forms throughout an advanced WWTP. The authors quantified estrogen concentrations in water and sludge samples and investigated also the estrogenic activity using estrogen-responsive reporter cell lines. Estrogen concentrations and estrogenicity measured in the inlet and in primary treated sewage showed a weak impact of primary treatment; however they observed a decrease of both estrogen concentration and estrogenicity during biological treatment. The removal was >90% of original concentration ranging between 200 and 500 ng/L. On the base of analysis of estrogens sorbed into the sludge they suggested that biodegradation was the main vehicle for estrogen elimination.

In spite of the application of very high initial concentrations (2.5×10^{-5} to 50 mg L^{-1}), experiments using bisphenol A, E2, and EE2, as well as activated and inactivated sludge, showed a high adsorption affinity of the compounds to the adsorbent (Clara et al., 2004). The adsorption in this work was also found to depend on pH, where the authors observed increasing solubility of adsorbed EE2 with elevated pH from 7 to 12. Correlation of EE2 sorption to activated sludge biomass and temperature was described (Xu et al., 2008).

The efficiency (>90%) of E1 and EE2 removal studied in municipal WWTPs under various redox conditions, demonstrated the importance of aerobic conditions for the removal of all estrogens (Joss et al., 2004). The importance of adaptation of microbial populations for the removal of estrogens (mainly E2), was shown by dramatic differences in mineralization of ^{14}C-labelled estrogenic compounds when working with biosolids from four municipal treatment plants and one industrial plant (Layton et al., 2000); in the municipal WWTP the mineralization was 70–80% during the 24 h, while in the industrial WWTP it reached only 4%. The mineralization of EE2 was 25–75-fold less than that of E2 and, in addition, it did not reach completion in 24 h, with only 40% mineralized to $^{14}CO_2$. Changes in temperature during the process significantly affected mineralization of E2 but had no effect on the rate of EE2 metabolism (Layton et al., 2000).

Several years of research have been focused on factors involved in the removal of estrogenic compounds from WWTPs, where the efficiency of the biological processes involved is highly dependent on operating parameters such as sludge retention time, redox potential, etc. – reviewed in Koh et al. (2008). Cao et al. (2008) reported a relationship between the equivalent biomass concentration and the estrogen degradation in WWTPs.

Under anaerobic conditions E1 was reduced to E2 but the extent of this reduction depended on the type of inocula (Mes et al., 2008). No significant loss of the sum of E1 and E2, or of EE2 was observed in activated sludge during the experiment under strictly anaerobic conditions. In the effluent, however, there was still a large fraction of E1 and E2 (adsorption to sludge accounted for a 32–35% loss from the liquid phase) but no EE2.

A work focused on potential relationship between the availability of oxygen, nitrification rate, and estrogen removal demonstrated that EE2 was persistent under anoxic conditions in activated sludge (Dytczak et al., 2008). Under aerobic conditions, the observed level of EE2 removal was 22% within 7 h. The higher removal of estrogens was associated with higher nitrification rate, thus nitrifying biomass could be responsible for their removal. The fate of E1, E2, and EE2 under different redox settings was also investigated in two nitrite-accumulating sequencing batch reactors, with different sludge ages operating under aerobic conditions and anoxic/anaerobic/aerobic conditions (Pholchan et al., 2008). The removal of EE2 under anaerobic conditions was considered to be mainly the result of sorption; however, the binding of estrogens to the sludge was apparently not as strong as the binding observed in the sludge under strictly aerobic conditions. Probably because of nitrite toxicity and the inhibition of the monooxygenase (AMO) and the microbial population generally, EE2 removal was adversely affected when the reactor operated with sludge retention time (SRT) shorter than 5.7 days and it was significantly lower when SRT was longer than 7.5 days. In contrast, during anaerobic digestion of estrogens by sewage sludge, no influence of temperature or SRT on the process was observed (Carballa et al., 2007).

Distribution coefficients (K_d) between water and activated sludge particles were used to estimate the fraction of the total estrogen concentration which is expected to be sorbed in the activated sludge under aerobic conditions (Andersen et al., 2005). Estimations based on the measured sorption constants predict that about 50–75% of steroid estrogens will be sorbed to activated sludge during activated sludge treatment in a WWTP.

Taking into account also the results of previous studies, the scenario of EE2 removal under aerobic conditions could be more mixed. Ternes et al. (1999) showed that E2 was oxidized and further eliminated by activated sludge from WWTP in Germany, while EE2 was principally persistent under selected conditions. Similarly, Weber et al. (2005) reported persistency of EE2 in batch experiments with activated sludge from two different types of WWTP. Further culture enrichment and isolation led to a defined mixed culture consisting of two strains, which were identified as *Achromobacter xylosoxidans* and *Ralstonia* sp. The culture used E1 and E2 as growth substrates, but not the EE2. In contrast, Forrez et al. (2009a) recorded 90% EE2 removal in an aerated nitrifying batch reactor and a complete biological EE2 removal from the synthetic effluent in a fixed bed reactor. They also demonstrated that once established nitrifiers could maintain the EE2 degradative activity, even without further addition of ammonium (Forrez et al., 2009a,b).

Cirja et al. (2007) studied the metabolism of two differently labelled radioactive forms of EE2 (concentration 8 g L^{-1}) in a membrane bioreactor (MBR), operating with 25 days SRT during the whole experimental period (35 days). Balancing of radioactivity demonstrated that the real mineralization was <1%, while radioactivity mainly remained sorbed in the reactor, resulting in a removal of approximately 80%. The result showed that the elimination pathway does not involve the removal of the ethinyl group from EE2 molecule. Despite its efficiency in bisphenol A removal, the additional disinfection of MBR effluent provided no further significant removal of E1, EE2, or E2 (Spring et al., 2007).

7. Algae

Only one publication is available dealing with biotransformation of EE2 by algae. Della Greca and co-workers (Della Greca et al., 2008) tested the biotransformation capability of 11 microalgae strains on EE2. Out of the tested strains, *Selenastrum capricornutum*, *Scenedesmus quadricauda*, *Scenedesmus vacuolatus*, and *Ankistrodesmus braunii* were able to biotransform the substrate. Several transformation products were identified (Fig. 3). These results are

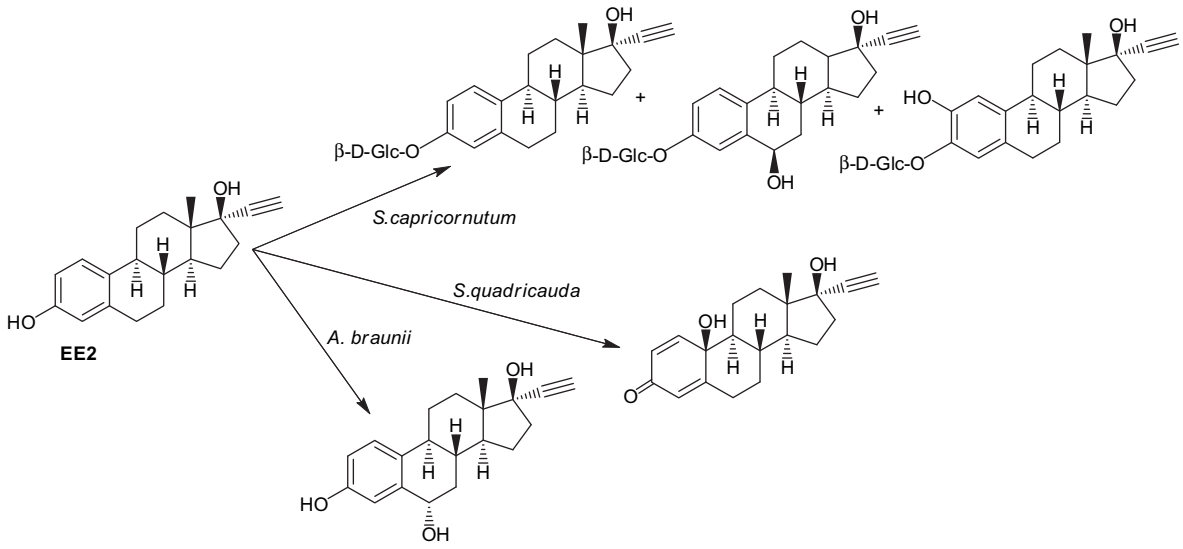

Fig. 3. Transformation products of EE2 by microalgae (Della Greca et al., 2008).

in agreement with previous studies on sterols and phenols that were degraded by *S. quadricauda* indicating the ability of the strain to perform regio- and stereo-selective hydroxylation of steroidic substrates (Della Greca et al., 1996, 1997). Whereas *A. braunii* showed another strategy during bioconversion of sinapic acid to 3,6-dihydroxy-2,4-dimethoxy-7H-benzocyclohepten-7-one via thomasidioic acid (Della Greca et al., 2003).

8. Conclusions

EDCs tend to accumulate in aquatic organisms and to be adsorbed on sediments and on particles in the aquatic environment. Due to their high bioactivity, ubiquitous nature, toxicity and persistence, it is of extreme importance to investigate and study organisms that have the ability to reduce these substances in the environment.

EE2 was found to be slowly decomposed by bacteria under anaerobic conditions. The dissipation time can exceed 1000 days and the degradation is attributed to sulfate-, nitrate-, and iron-reducing conditions. However, abiotic factors can also play an important role in the removal. Faster degradation can be recorded for dissolved EE2 by bacteria under aerobic conditions and also seawater microbes were found to degrade EE2 after acclimation. More rapid degradation of EE2 was also linked to the presence of AOM bacteria from activated sludge. EE2 was found to be more resistant to bacterial biodegradation than natural estrogens, but its highly hydrophobic nature makes sorption a significant removal factor in WWTPs. Using also batch experiments, it was found that WWTP processes based on activated sludge are more effective in the EE2 removal; however, the results are strongly dependent on the operating parameters (e.g. temperature, SRT, redox potential etc.) and only few authors have so far evaluated changes in estrogenic activity in the WWTP process.

Another promising alternative for EE2 decomposition could be an application of ligninolytic fungi. A number of fungal strains were shown to degrade efficiently EE2 and other estrogens, and also a direct application of ligninolytic enzymes was proved to be successful in the EE2 degradation within a relatively short time. These systems were also able to remove estrogenic activities of the samples.

Recently, several studies have been performed to evaluate the persistency of EE2 in soil. Although some of the works showed apart from sorption also possible microbial degradation, the fate of EE2 in soil remains unclear.

As yet, the mechanisms of these degradations have not been elucidated and only several authors detected EE2 transformation products from fungi, bacteria and microalgae. The conclusions of this review therefore emphasize the need for further research and the future tasks that emerge from the data gatherered here can be summarized as follows:

1. Search for microbial (bacteria, white rot fungi) species and strains with high (either compound-specific or general) estrogen-degrading activity and their use in wastewater treatment in a way that would allow a flexible and efficient degradation;
2. Study of degradative reactions and pathways that do not yield metabolites with estrogenic activity;
3. Investigations of material matrices and conditions ensuring efficient abiotic removal of estrogens (hydrolysis, chemical reduction, photolysis, sorption or volatilization);
4. Optimization of the application of enzymes in degredative processes;
5. Designing WWTP systems that would integrate all the above factors.

Acknowledgements

This work was funded by grant No. LC06066 of the Ministry of Education, Youth and Sport of the Czech Republic, by grant KJB600200613 of the Grant Agency of the Academy of Science of the Czech Republic; and also by Institutional Research Concept No. AV0Z50200510.

References

Aerni, H.R., Kobler, B., Rutishauser, B.V., Wettstein, F.E., Fischer, R., Giger, W., Hungerbuhler, A., Marazuela, M.D., Peter, A., Schonenberger, R., Vogeli, A.C., Suter, M.J.-F., Eggen, R.I.L., 2004. Combined biological and chemical assessment of estrogenic activities in wastewater treatment plant effluents. Analytical and Bioanalytical Chemistry 378, 688–696.

Andersen, H.R., Hansen, M., Kjolholt, J., Stuer-Lauridsen, F., Ternes, T., Halling-Sorensen, B., 2005. Assessment of the importance of sorption for steroid estrogens removal during activated sludge treatment. Chemosphere 61, 139–146.

Auriol, M., Filali-Meknassia, Y., Tyagi, R.D., Adams, C.D., 2007a. Laccase-catalyzed conversion of natural and synthetic hormones from a municipal wastewater. Water Research 41, 3281–3288.

Auriol, M., Filali-Meknassi, Y., Adams, C.D., Tyagi, R.D., Noguerol, T.N., Pin, B., 2008. Removal of estrogenic activity of natural and synthetic hormones from a municipal wastewater: efficiency of horseradish peroxidase and laccase from *Trametes versicolor*. Chemosphere 70, 445–452.

Auriol, M., Filali-Meknassia, Y., Adams, C.D., Tyagi, R.D., 2006. Natural and synthetic hormone removal using the horseradish peroxidase enzyme: temperature and pH effects. Water Research 40, 2847–2856.

Auriol, M., Filali-Meknassia, Y., Tyagi, R.D., Adams, C.D., 2007b. Oxidation of natural and synthetic hormones by the horseradish peroxidase enzyme in wastewater. Chemosphere 68, 1830–1837.

Blánquez, P., Guieysse, B., 2008. Continuous biodegradation of 17β-estradiol and 17α-ethynylestradiol by *Trametes versicolor*. Journal of Hazardous Material 150, 459–462.

Braga, O., Smythe, G.A., Schafer, A.I., Feltz, A.J., 2005a. Steroid estrogens in primary and tertiary wastewater treatment plants. Water Science and Technology 52, 273–278.

Braga, O., Smythe, G.A., Schafer, A.I., Feitz, A.J., 2005b. Fate of steroid estrogens in Australian inland and coastal wastewater treatment plants. Environmental Science & Technology 39, 3351–3358.

Cao, Q., Yu, Q., Connell, D.W., 2008. Degradation rate constants of steroids in sewage treatment works and receiving water. Environmental Technology 29, 1321–1330.

Cajthaml, T., Křesinová, Z., Svobodová, K., Möder, M., 2009. Biodegradation of endocrine disrupting compounds and suppression of estrogenic activity by ligninolytic fungi. Chemosphere 75, 745–750.

Carballa, M., Omil, F., Ternes, T., Lema, J.M., 2007. Fate of pharmaceutical and personal care products (PPCPs) during anaerobic digestion of sewage sludge. Water Research 41, 2139–2150.

Choudhary, M.I., Musharraf, S.G., Ali, R.A., Atif, M., Atta-ur-Rahman, 2004. Microbial transformation of Antifertility agents, norethisterone and 17-α-ethinyl estradiol. Zeitschrift für Naturforschung 59b, 319–323.

Cirja, M., Zuehlke, S., Ivashechkin, P., Hollender, J., Schaffer, A., Corvini, P.F.X., 2007. Behavior of two differently radiolabelled 17α-ethinylestradiols continuously applied to a laboratory-scale membrane bioreactor with adapted industrial activated sludge. Water Research 41, 4403–4412.

Clara, M., Strenn, B., Saracevic, E., Kreuzinger, N., 2004. Adsorption of bisphenol-A, 17 beta-estradiole and 17α-ethinylestradiole to sewage sludge. Chemosphere 56, 843–851.

Clouzot, L., Marrot, B., Doumenq, P., Roche, N., 2008. 17α-ethinylestradiol: an endocrine disrupter of great concern. Analytical methods and removal processes applied to water purification. A review. Environmental Progress 27, 383–396.

Colborn, T., Saal, F.S.V., Soto, A.M., 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Evironmental Health Perspectives 101, 378–384.

Colucci, M.S., Topp, E., 2001a. Persistence of estrogenic hormones in agricultural soils: I. 17β-estradiol and estrone. Journal of Environmental Quality 30, 2070–2076.

Colucci, M.S., Topp, E., 2001b. Persistence of estrogenic hormones in agricultural soils: II. 17α-ethynylestradiol. Journal of Environmental Quality 30, 2077–2080.

Czajka, C.P., Londry, K.L., 2006. Anaerobic biotransformation of estrogens. Science of the Total Environment 367, 932–941.

Della Greca, M., Pinto, G., Pistillo, P., Pollio, A., Previtera, L., Temussi, F., 2008. Biotransformation of ethinylestradiol by microalgae. Chemosphere 70, 2047–2053.

Della Greca, M., Fiorentino, A., Guerriero, I., Pinto, G., Pollio, A., Previtera, L., 1997. Biotransformation of adrenosterone into 11- ketotestosterone by *Scenedesmus quadricauda* grown in myxotrophic conditions. Biotechnology Letters 19, 1123–1124.

Della Greca, M., Fiorentino, A., Pinto, G., Pollio, A., Previtera, L., 1996. Biotransformation of progesterone by the green alga *Chlorella emersonii* C211-8H. Phytochemistry 41, 1527–1529.

Della Greca, M., Pinto, G., Pollio, A., Previtera, L., Temussi, F., 2003. Biotransformation of sinapic acid by the green algae *Stichococcus bacillaris* 155LTAP and Akistrodesmus braunii C202. 7a. Tetrahedron Letters 44, 2779–2780.

Dytczak, M.A., Londry, K.L., Oleszkiewicz, J.A., 2008. Biotransformation of estrogens in nitrifying activated sludge under aerobic and alternating anoxic/aerobic conditions. Water Environment Research 80, 47–52.

Forrez, I., Carballa, M., Boon, N., Verstraete, W., 2009a. Biological removal of 17a-ethinylestradiol (EE2) in aerated nitrifying fixed bed reactor during ammonium starvation. Journal of Chemical Technology and Biotechnology 84, 119–125.

Forrez, I., Carballa, M., Noppe, H., De Brabander, H., Boon, N., Verstraete, W., 2009b. Influence of manganese and ammonium oxidation on the removal of 17a-ethinylestradiol (EE2). Water Research 43, 77–86.

Fujii, K., Kikuchi, S., Satomi, M., Ushio-Sata, N., Morita, N., 2002. Degradation of 17 beta-estradiol by a gram-negative bacterium isolated from activated sludge in a sewage treatment plant in Tokyo, Japan. Applied and Environmental Microbiology 68, 2057–2060.

Gaulke, L.S., Strand, S.E., Kalhorn, T.F., Stensel, H.D., 2008. 17α-ethinylestradiol transformation via abiotic nitration in the presence of ammonia oxidizing bacteria. Environmental Science and Technology 42, 7622–7627.

Gutendorf, B., Westendorf, J., 2001. Comparison of an array of in vitro assays for the assessment of the estrogenic potential of natural and synthetic estrogens, hytoestrogens and xenoestrogens. Toxicology 166, 79–89.

Horinouchi, M., Hayashi, T., Yamamoto, T., Kudo, T., 2003. A new bacterial steroid degradation gene cluster in *Comamonas testosteroni* TA441 which consist of aromatic-compound degradation genes for seco-steroids and 3-ketosteroid dehydrogenase genes. Applied Microbiology and Biotechnology 69, 4421–4430.

Johnson, A.C., Aerni, H.R., Gerritsen, A., Gibert, M., Giger, W., Hylland, K., Jurgens, M., Nakari, T., Pickering, A., Suter, M.J.F., Svenson, A., Wettstein, F.E., 2005. Comparing steroid estrogen, and nonylphenol content across a range of European sewage plants with different treatment and management practices. Water Research 39, 47–58.

Johnson, A.C., Sumpter, J.P., 2001. Removal of endocrine-disrupting chemicals in activated sludge treatment works. Environmental Science & Technology 35, 4697–4703.

Joss, A., Andersen, H., Ternes, T., Richle, P.R., Siegrist, H., 2004. Removal of estrogens in municipal wastewater treatment under aerobic and anaerobic conditions: consequences for plant optimization. Environmental Science & Technology 38, 3047–3055.

Jurgens, M.D., Holthaus, K.I.E., Johnson, A.C., Smith, J.J.L., Hetheridge, M., Williams, R.J., 2002. The potential for estradiol and ethinylestradiol degradation in English rivers. Environmental Toxicology and Chemistry 21, 480–488.

Kanda, R., Churchley, J., 2008. Removal of endocrine disrupting compounds during conventional wastewater treatment. Environmental Technology 29, 315–323.

Ke, J.X., Zhuang, W.Q., Gin, K.Y.H., Reinhard, M., Hoon, L.T., Tay, J.H., 2007. Characterization of estrogen-degrading bacteria isolated from an artificial sandy aquifer with ultrafiltered secondary effluent as the medium. Applied Microbiology and Biotechnology 75, 1163–1171.

Koh, Y.K.K., Chiu, T.Y., Boobis, A., Cartmell, E., Scrimshaw, M.D., Lester, J.N., 2008. Treatment and removal strategies for estrogens from wastewater. Environmental Technology 29, 245–267.

Kwak, H.I., Bae, M.O., Lee, M.H., Lee, Y.S., Lee, B.J., Kang, K.S., Chae, C.H., Sung, H.J., Shin, J.S., Kim, J.H., Mar, W.C., Sheen, Y.Y., Cho, M.H., 2001. Effects of nonylphenol, bisphenol A, and their mixture on the viviparous swordtail fish (*Xiphophotus helleri*). Environmental Toxicology and Chemistry 20, 787–795.

Layton, A.C., Gregory, B.W., Seward, J.R., Schultz, T.W., Sayler, G.S., 2000. Mineralization of steroidal hormones by biosolids in wastewater treatment systems in Tennessee USA. Environmental Science & Technology 34, 3925–3931.

Lorenzen, A., Burnison, K., Servos, M., Topp, E., 2006. Persistence of endocrine disrupting chemicals in agricultural soils. Journal of Environmental Engeneering Ans Science 5, 211–219.

Lugaro, G., Carrea, G., Cremonesi, P., Casellato, M.M., Antonini, E., 1973. The oxidation of steroid hormones by fungal laccase in emulsion of water and organic solvents. Archives of Biochemistry and Biophysics 1, 1–6.

Matsuoka, S., Kikuchi, M., Kimura, S., Kurokawa, Y., Kawai, S., 2005. Determination of estrogenic substances in the water of Muko River using in vitro assays, and the degradation of natural estrogens by aquatic bacteria. Journal of Health Science 51, 178–184.

Mes, T.Z.D.D., Kujawa-Roeleveld, K., Zeeman, G., Lettinga, G., 2008. Anaerobic biodegradation of estrogens – hard to digest. Water Science and Technology 57, 1177–1182.

Miège, C., Choubert, J.M., Ribeiro, L., Eusèbe, M., Coquery, M., 2009. Fate of pharmaceuticals and personal care products in wastewater treatment plants – conception of a database and first results. Environmental Pollution 157, 1721–1726.

Muller, M., Rabenoelina, F., Balaguer, P., Patureau, D., Lemenach, K., Budzinsky, H., Barcelo, D., De Alda, M.L., Kuster, M., Delgenes, J.P., Hernandez-Raquet, G., 2008. Chemical and biological analysis of endocrine-disrupting hormones and estrogenic activity in an advanced sewage treatment plants. Environmental Toxicology and Chemistry 27, 1649–1658.

Nicotra, S., Intra, A., Ottolina, G., Riva, S., Danieli, B., 2004. Laccase-mediated oxidation of the steroid hormone 17β-estradiol in organic solvents. Tetrahedron-Asymmetry 15, 2927–2931.

Pauwels, B., Noppe, H., De Branbander, H., Verstraete, W., 2008a. Comparison of streriod hormone concentration in domestic and hospital wastewater treatment plants. Journal of Environmental Engeneering – ASCE 134, 933–936.

Pauwels, B., Wille, K., Noppe, H., De Brabander, H., de Wiele, T.V., Verstraete, W., Boon, N., 2008b. 17α-ethinylestradiol cometabolism by bacteria degrading estrone, 17β-estradiol and estriol. Biodegradation 19, 683–693.

Pholchan, P., Jones, M., Donnelly, T., Sallis, P.J., 2008. Fate of estrogens during the biological treatment of synthetic wastewater in a nitrite-accumulating sequencing batch reactor. Environmental Science & Technology 42, 6141–6147.

Purdom, C.E., Hardiman, P.A., Bye, V.J., Eno, N.C., Tyler, C.R., Sumpter, J.P., 1994. Estrogenic effects of effluents from sewage treatment works. Chemistry and Ecology 8, 275–285.

Ren, H.Y., Ji, S.L., Ahmad, N.U.D., Dao, W., Cui, C.W., 2007a. Degradation characteristics and metabolic pathway of 17α-ethynylestradiol by *Sphingobacterium* sp. JCR5. Chemosphere 66, 340–346.

Ren, Y.X., Nakano, K., Nomura, M., Chiba, N., Nishimura, O., 2007b. Effects of bacterial activity on estrogen removal in nitrifying activated sludge. Water Research 41, 3089–3096.

Sarmah, A.K., Northcott, G.L., 2008. Laboratory degradation studies of four endocrine disruptors in two environmental media. Environmental Toxicology and Chemistry 27, 819–827.

Shi, J., Fujisawa, S., Nakai, S., Hosomi, M., 2004. Biodegradation of natural and synthetic estrogens by nitrifying activated sludge and ammonia-oxidizing bacterium *Nitrosomonas europaea*. Water Research 38, 2323–2330.

Shi, J.H., Suzuki, Y., Lee, B.D., Nakai, S., Hosomi, M.I., 2002. Isolation and characterization of the ethynylestradiol-biodegrading microorganism *Fusarium proliferatum* strain HNS-1. Water Science and Technology 45, 175–179.

Soto, A.M., Justicia, H., Wray, J.W., Sonnenschein, C., 1991. P-nonylphenol: an estrogenic xenobiotic released from modified polystyrene. Environmental Health Perspectives 92, 167–173.

Spring, A.J., Bagley, D.M., Andrews, R.C., Lemanik, S., Yang, P., 2007. Removal of endocrine disrupting compounds using a membrane bioreactor and disinfection. Journal of Environmental Engineering and Science 6, 131–137.

Stumpe, B., Marschner, B., 2009. Factors controlling the biodegradation of 17β-estradiol, estrone, and 17α-ethinylestradiol in different natural soils. Chemosphere 74, 556–562.

Suzuki, K., Hiraia, H., Muratab, H., Nishidaa, T., 2003. Removal of estrogenic activities of 17b-estradiol and ethinylestradiol by ligninolytic enzymes from white rot fungi. Water Research 37, 1972–1975.

Tamagawa, Y., Yamaki, R., Hirai, H., Kawai, S., Nishida, T., 2006. Removal of estrogenic activity of natural steroidal hormone estrone by ligninolytic enzymes from white rot fungi. Chemosphere 65, 97–101.

Tanaka, T., Tonosaki, T., Nose, M., Tomidokoro, N., Kadomura, N., Fujii, T., Taniguchi, M., 2001. Treatment of model soils contaminated with phenolic endocrine-disrupting chemicals with laccase from Trametes sp in a rotating reactor. Journal of Bioscience and Bioengineering 92, 312–316.

Ternes, T.A., Kreckel, P., Mueller, J., 1999. Behaviour and occurrence of estrogens in municipal sewage treatment plants - II. Aerobic batch experiments with activated sludge. Science of the Total Environment 225, 91–99.

Tyler, C.R., Routledge, E.J., 1998. Oestrogenic effects in fish in English rivers with evidence of their causation. Pure and Applied Chemistry 70, 1795–1804.

Vader, J.S., van Ginkel, C.G., Sperling, F.M.G.M., de Jong, J., de Boer, W., de Graaf, J.S., van der Most, M., Stokman, P.G.W., 2000. Degradation of ethinyl estradiol by nitrifying activated sludge. Chemosphere 41, 1239–1243.

Weber, S., Leuschner, P., Kampfer, P., Dott, W., Hollender, J., 2005. Degradation of estradiol and ethinyl estradiol by activated sludge and by a defined mixed culture. Applied Microbiology and Biotechnology 67, 106–112.

Xu, K., Harper Jr., W.F., Zhao, D., 2008. 17α-Ethinylestradiol sorption to activated sludge biomass: thermodynamic properties and reaction mechanism. Water Research 42, 3146–3152.

Yi, T., Harper, W.F., 2007. The link between nitrification and biotransformation of 17 alpha-ethinylestradiol. Environmental Science & Technology 41, 4311–4316.

Yi, T.W., Harper, W.F., Holbrook, R.D., Love, N.G., 2006. Role of particle size and ammonium oxidation in removal of 17 alpha-ethinyl estradiol in bioreactors. Journal of Environmental Engineering-ASCE 132, 1527–1529.

Ying, G.G., Kookana, R.S., 2003. Degradation of five selected endocrine-disrupting chemicals in seawater and marine sediment. Environmental Science & Technology 37, 1256–1260.

Ying, G.G., Kookana, R.S., Dillon, P., 2003. Sorption and degradation of selected five endocrine disrupting chemicals in aquifer material. Water Research 37, 3785–3791.

Ying, G.G., Kookana, R.S., Kumar, A., 2008b. Fate of estrogens and xenoestrogens in four sewage treatment plants with different technologies. Environmental Toxicology and Chemistry 27, 87–94.

Ying, G.G., Toze, S., Hanna, J., Yu, X.Y., Dillon, P.J., Kookana, R.S., 2008a. Decay of endocrine-disrupting chemicals in aerobic and anoxic groundwater. Water Research 42, 1133–1141.

Yoshimoto, T., Nagai, F., Fujimoto, J., Watanabe, K., Mizukoshi, H., Makino, T., Kimura, K., Saino, H., Sawada, H., Omura, H., 2004. Degradation of estrogens by *Rhodococcus zopfii* and *Rhodococcus equi* isolates from activated sludge in wastewater treatment plants. Applied and Environmental Microbiology 70, 5283–5289.