Environmental Engineering and Management Journal April 2018, Vol.17, No. 4, 977-998
http://www.eemj.icpm.tuiasi.ro/; http://www.eemj.eu

"Gheorghe Asachi" Technical University of Iasi, Romania

FATE AND BIODEGRADATION OF ESTROGENS IN THE ENVIRONMENT AND ENGINEERING SYSTEMS – A REVIEW

Hai-Liang Song[1,2]*, Shi-Bei Huang[2], Xiao-Li Yang[3]

[1]*School of Environment, Nanjing Normal University, Nanjing 210023, China*
[2]*School of Energy and Environment, Southeast University, Nanjing 210096, China*
[3]*School of Civil Engineering, Southeast University, Nanjing 210096, China*

Abstract

Estrogens excreted by humans and animals are groups of endocrine disruptors, which degrade rapidly in soil and water as reported. This review focuses on biodegradation of estrone (E1), 17β-estradiol (E2) and 17α-ethynylestradiol (EE2) in surface water, STPs (sewage treatment plants), manure, soil and sediments, and illustrates possible pathways and mechanisms. In general, half-lives of EE2 are much longer than E1 and E2, because an ethinyl group at one hydroxyl group containing C-atom makes the cleavage of this ring rather difficult. Thus, EE2 has great estrogenic potential although the secreted amount is much smaller than that of E2 or E1. Various kinds of bacteria and fungus, *Cornybacterium* spp., *Nitrosomonas europaea* etc., are reported to be capable of degrading estrogens. Moreover, reports indicate that temperature, pH values etc. exert impacts on degradation to different extents. The biodegradation in the sludge phase was assumed by researchers to follow a pseudo-first-order reaction, and the sequence of *K*-values is E2>E1>>EE2 for the same sludge. As for the pathways, it was found that E2 is oxidized to E1 by the first step. The half-live of this step is about 4 to 12 hours in aerobic water and soil. However, this step cannot remarkably reduce the estrogenic potential. Further degradation of E1 needs the cleavage of one ring. Therefore, half-lives and concentrations of E1 are much longer and higher than those of E2. As a matter of fact, pathways of EE2 are still controversial, as several incompatible theories have been proposed.

Key words: biodegradation, co-metabolism, endocrine disruption, estrogen

Received: July, 2013; Revised final: July, 2014; Accepted: July, 2014; Published in final edited form: April 2018

1. Introduction

Research about the occurrence and fate of estrogenic compounds in wastewater emerged in the mid-1990s (Anderson et al., 2003; Kumar et al., 2016; Matsui et al., 2000; Ternes et al., 1999a). In general, there are two kinds of estrogenic compounds, endogenous estrogens and xenoestrogens respectively. Human and animal waste-borne steroidal hormones, also called natural steroidal estrogens, belong to the group of endogenous steroidal Endocrine-Disrupting Compounds (EDCs), which have high estrogenic potency. These steroids have been detected in the effluent of sewage treatment plants (STPs) and surface water (Desbrow et al., 1998; Kuch and Ballschmiter, 2001; Preda et al., 2011; Ternes et al., 1999a). Among the steroids, three sterols, the natural hormones 17β-estradiol (E2), estrone (E1) and the synthetic hormone 17α-ethynylestradiol (EE2), were isolated from the effluents of domestic STPs and identified as a dominating contributor to its estrogenic character (Chen et al., 2016; Desbrow et al., 1998; Körner et al., 2001; Onda et al., 2003; Routledge et al., 1998). Research has been conducted to quantify human-excreted estrogens in raw sewage and in treated effluent. It is found that natural estrogen hormones excreted by humans, e.g. 17α-estradiol, have levels as

* Author to whom all correspondence should be addressed: e-mail: hlsong@njnu.edu.cn; Phone:+86 2585891849; Fax:+86 25 85891455

high as 5 mg/day for pregnant women. The estrogen (particularly 17α-estradiol, estrone, and estriol) concentrations in the effluent of a conventional sewage treatment plant (STP) range from a few nanograms per liter (ng/L) to several μg/L.

These micropollutants, which are at relatively low levels compared with other pollutants, have properties of EDCs which may modify diverse physiological functions, e.g. reproduction and development in different species including fish and humans (Nakamura et al., 2014; Sumpter et al., 1998; Zuo et al., 2013). Recent studies point out that estrogens affect the gene expression in fish (Joseph et al., 2014) and increase breast cancer risk in both exposed mothers and their daughters if they are exposed to synthetic estrogens during pregnancy (Hilakivi et al., 2013).

With the measurements by the MELN cell line in vitro test, the relative estrogenic activity for different EDCs, with the E2 estrogenicity arbitrarily fixed at 100, display values of 246 for EE2, 17.6 for estriol (E3), 2.5 for E1 (Balaguer et al., 1999; Pillon et al., 2005; Sarah and Guillermina, 2010). Such great estrogenic activity found in hormones underlines their possible negative impact on the natural environment.

Biodegradation has been reported the major removal mechanism that affects the fate and transfer of estrogenic compounds in the environment and engineering systems (Johnson and Sumpter, 2001; Writer et al., 2011). This article is mainly aimed to review the recent findings with respect to biodegradation pathways and mechanisms of estrogens, e.g., E1, E2, E3, EE2, in different phases and systems. Relevant background information, such as structure, physicochemical properties, sources, occurrences, and analytical techniques, is also briefly discussed.

2. Synthesis, structure and properties of estrogens

The principal estrogens of environmental concern are naturally occurring estrogens: estrone (E1), estradiol (E2), and estriol (E3) (Sarah and Guillermina, 2010), as well as a synthetic estrogen ethinylestradiol (EE2), which is used in the formulation of the contraceptive pill.

In general, estrogens dissolve poorly in water, as evidenced by Lai et al. (2000), with solubility of about 13 mg/L for the natural compounds, while EE2 display solubility of 4.8 mg/L, which is even lower. The octanol - water partition (K_{ow}) of the estrogens is between 2.8 and 4.2 (Lai et al., 2000). Both the vapor pressure and the volatility of these estrogens are relatively low. The relative estrogenic activity of E2 is around 100, while that of EE2 is as high as 246.

3. Estrogen sources and levels in the environment

Estrogens produced by human glands are excreted within urine and faeces. The synthetic EE2 is used in oral contraceptives world-wide (Williams and Stancel, 1996). Liver of humans and animals is the place where estrogens are enzymatically mediated and conjugated with either sulphate or glucuronide esters to the hydroxyl groups in the C3- and C7-position of the basic steroid structure (Williams and Stancel, 1996). Conjugation boosts the water solubility, which makes estrogens more mobile in the environment than free hormones. Natural hormones excreted in faeces, which occupies a very small proportion of effluent hormones, are mainly in an unconjugated form. The reason for the estrogens being unconjugated in faeces is the capability of bacteria, e.g. *E.coli*, to produce the enzyme β-glucuronidase, which can hydrolyse glucuronide conjugates back to their original form (Legler 2001; Ternes et al., 1999a).

To our knowledge, high performance liquid chromatography (HPLC) and gas chromatography-mass spectrometer (GC-MS) are appropriate for the determination of estrogens. Various preconcentration methods have been established, as well as other detection methods Zou et al. (2013) reported hollow-fiber-supported liquid-phase microextraction using an ionic liquid as the extractant for the preconcentration of estrogens from water samples with HPLC detection. Li et al. (2013a) optimized the solid-phase extraction for determining estrone in sewage. In addition, Owen and Keevil (2013) describe a sensitive and rapid Liquid Chromatography-Mass Spectrometry (LC-MS/MS) assay for the simultaneous measurement of serum estradiol and estrone, which is similar to the methods established by Szarka et al. (2013).

Table 1. Structure and properties of natural and synthetic steroidal estrogen hormones

Compound	*Chemical structure*	*Molecular weight (g/mol)*	*Water solubility[a] (mg/L)*	*Log Kow[b]*	*Vapor pressure (mmHg)[c]*	*Relative estrogenic activity[d]*
Estrone(E1)		270.4	13	3.43	2.3×10^{-10}	2.54

17β-estradiol(E2)		272.4	13	3.94	2.3×10^{-10}	100
Estriol(E3)		288.4	13	2.81	6.7×10^{-15}	17.6
17α-Ethinylestradiol(EE2)		296.4	4.8	4.15	4.5×10^{-11}	246

a - solubility in water at 20°C (Lai et al., 2000); b - octanol-water partition coefficient (Lai et al., 2000); [c](Lai et al., 2000); [d] (Pillon et al., 2005); MELN in vitro test

Table 2. Daily values of E1, E2 and E3 in urine and faece excreted by women

Form	***Carriers***	***E1(µg)***	***E2(µg)***	***E3(µg)***	***References***
unconjugated	urine	8.00	3.00	4.80	Fotsis et al., 1980
	urine	7.00	2.40	4.60	Adlercreutz et al., 1986
	faece	0.50	0.40	1.25	Adlercreutz et al., 1994

According to Williams and Stancel (1996), the total daily excretion of natural estrogens ranges from 10 to 100 µg for women, 5 to 10 µg for women after the menopause and 2 to 25 µg for men. Average excretion values of unconjugated natural estrogens and the synthetic EE2 were tested, as shown in Table 2. From Adlercreutz et al. (1986), women can excrete 7 µg of E1, 2.4 µg of E2, and 4.6 µg of E3 of unconjugated forms daily via urine. Approximately 0.4 µg E2, 1.25 µg of E3 and 0.5 µg E1 was excreted in faeces per day (Adlercreutz et al., 1994). Fotsis et al. (1980) reported a daily excretion in urine of unconjugated forms as 3.0 µg of E2, 8.0 µg of E1 and 4.8 µg of E3. In addition, Johnson et al. (2000) reported that male excreted 1.6 µg/day of E2, 3.9 µg/day of E1 and 1.5 µg/day of E3 in their urine. With that, the amount of E2 is relatively lower than E1 and E3. Additionally, the daily intake of EE2 ranges about 20-60 µg for contraception while approximately 10 µg to control menopausal disorders. This means that almost 30-90 percent of EE2 is excreted in urine and feces (Johnson and Williams 2004; Sarah and Guillermina, 2010; Webb et al., 2003).

As for animals, E1, E2, and E3 are the main parts of natural estrogens excretion (Sarmah et al., 2006). The daily excretion of estrogens changes with animal species, sex, circadian rhythm, and reproductive state (Lange et al., 2001). The wide differences between species were evidenced by radiotracer studies, which showed that cattle excreted estrogens mostly in faeces (58%), whereas swine and poultry excreted estrogens mostly in urine (96% and 69%, respectively) (Ainsworth et al., 1962; Ivie et al., 1986; Palme et al., 1996). However, the ratio fluctuates during pregnancy (Hoffmann et al., 1997). Since urine and feces are not usually disposed separately in commercial animal production systems, the route of excretion would not appear to be an important environmental concern (Knight et al., 1980).

Estrogenic steroids have been measured in both STPs and surface water. In effluents of British STPs, the concentration of E1 and E2 ranged from 1 ng/L to almost 50-80 ng/L. The concentration of EE2 was generally below the limit of detection but was positively identified in three of the effluent samples at concentrations ranging from 0.2 to 7.0 ng/L (Desbrow et al., 1998). The concentrations reached 70 ng/L for E1, 64 ng/L for E2, 18 ng/L for E3 and 42 ng/L for EE2 in the effluents of sewage treatment plants (STPs) in different countries (Ying et al., 2002). In German river water, the concentrations reached 4.1 ng/L for E1, 3.6 ng/L for E2, and 5.1 ng/L for EE2 (Kuch and Ballschmiter, 2001). E2 reaches 27 ng/L in river waters from Japan, Italy, Germany and the Netherlands (Ying et al., 2002). Besides, E2 concentration measured in mantled karst aquifers in northwest Arkansas ranged from 6 to 66 ng/L (Peterson et al., 2000). In Lake Quinsigamond (Massachusetts, USA), EE2 was detected at a concentration up to 11.1 ng/L (Zuo et al., 2013). In wastewater from five sewage treatment plants (STPs) in Guangdong Province, China, E1 and E2 were detected in all influent samples at concentrations of

69.3–280 ng/L and 1.3–30 ng/L, respectively (Xu et al., 2014).

4. Degradation efficiency in the environment and in the engineered system

The biodegradation of unconjugated estrogens has been studied in soil, water, and manure for several years. It is obvious that biodegradation is not only an important process to remove estrogens in raw sewages of STPs, but also an enhancement to eliminate estrogens in the environment, thus reducing their risk via surface runoff and/or leaching. One key factor for hydrocarbon degradation in polluted habitats is the presence of an adequate electron acceptor (Berthe-Corti and Nachtkamp, 2010).

However, natural environments such as wet soils, swamps, fresh water, marine sediments and aquifers, are places where oxygen is often limited.

4.1. Degradation in surface water

Lai et al. (2002) reported that common freshwater algae (*Chlorella vulgaris*) were capable of oxidizing 17*β*-estradiol to estrone. Jürgens et al. (2002) observed similar degradation rates for E2 in river water for a spiking concentration range of 20, 50 and 100 ng/L.

They suggested that even when insufficient E2 was present to stimulate the multiplication of bacteria, E2 were still transformed. Degradation studies carried out in waters from five English rivers indicate that E2 has a half-life of 3-27 days. E1 is the first degradation product of E2 but no investigations of the subsequent by-products has been conducted. The poorest degradation rate was observed in the estuary river water samples, where the high salt content might inhibit microbial degradation. Jürgens et al. (2002) reported that the half-lives of estradiol and estrone at 20°C ranged from 0.2 to 9 d and from 0.1 to 11 d, respectively. Temperature may have a significant impact on degradation efficiency. Shortest half-lives from 4 to 5 hours were found for the degradation of E2 in summer at 20°C (cf. longest half-lives around 40 hours), while half-lives in winter and spring were 2.5 to 8.5 times higher than in summer (also tested at 20°C). Kuster et al. (2004) pointed out that EE2 (half-life, 46 days) is more reluctant than E2 (half-life 4 days, e.g., in the River Thames). These half-life values have possible reasons corresponding to ideal summer temperatures. However, under winter conditions, these compounds could be twice as persistent. Explanation is that higher prior temperatures in summer or higher nutrient concentrations due to less dilution in summer, which means that microorganisms exposed to the test system have higher initial activity.

EE2 was studied with a long half-life up to 17 days, which was much longer than E2. (Jürgens et al., 2002). In other words, EE2 was more recalcitrant in the water. According to de Mes et al. (2005), the structure of EE2 is the critical factor of such characteristic, as EE2 has an ethinyl group at position 17 which hinders the oxidation at this C-atom. Hence, EE2 exerts significant impact on the environment due to its stability in spite of its low concentration. Another way how EE2 can be degraded is by photolysis. The half-life was estimated by Jürgens et al. (2002) as 124 h for E2 and 126 h EE2, respectively (at 12 hours sunshine per day). The photolysis is slower than biodegradation, although EE2 removal through photolysis may be more significant at places where the sun is often shining. Contrary to this notion, a case study conducted by Zuo et al. (2013) showed that the photodegradation of EE2 was faster than biodegradation in the lake surface water under sunlight, with a half-life of less than 2 days in summer sunny days, which means photodegradation may represent a dominant removal mechanism for EE2 in natural surface waters. In that study, under aerobic conditions, a half-life of 108 days was estimated. Under anaerobic conditions, microbial degradation in the lake water was even slower. However, photolysis of estrone generates estrogenic photoproducts with higher activity than the parent compound (Souissi et al., 2014).

4.2. Degradation in soils and sediments

Compared to the biodegradation by sewage microbes, which is fast and complete, biodegradation by soil microbes is rather slow and incomplete. Sediments may serve as both a sink and a source of more estrogenic compounds, as reported by Mashtare et al. (2013).

Das et al. (2004) examined the sorption and degradation of E2 during transport by column studies, packed with surface soil, freshwater sediment and two sands, utilizing model evaluation. Degradation-rate coefficient (k) is in the range 0.0003-0.075/h for E2 and the rate for the only metabolite found (E1) was 1-2 orders of magnitude larger.

Colucci et al. (2001a) studied the mineralization of ^{14}C-E2 in loam, sandy loam, and silt loam soils from Canada. Only 11.5-17.1% of 1 mg/kg ^{14}C-E2 was evolved as $^{14}CO_2$ after 3 months of incubation at 30°C. Similarly, the mineralization efficiency of ^{14}C-E2 measured in terms of carbon dioxide released did not exceed 10% in both loam and silt soils with an initial concentration of 81.7μg/kg after 96 h of incubation (Jacobsen et al., 2005). Stumpe and Marschner (2007) reported that the mineralization of 1 mg/kg ^{14}C-E2 in four agricultural topsoils with different soil properties and different site histories like wastewater irrigation and sewage sludge application reached 5.1-7.4% during incubations at 20°C over three weeks. Apart from mineralization to $^{14}CO_2$, most of the applied estrogens were incorporated into "bound" (i.e. non-extractable) residues. It has been suggested that the non-extractable residues were relatively nonbioavailable (Colucci et al., 2001b), but characterization of the unknown products is necessary. As expected, EE2 was found more persistent than E2 in aerobic experiments with river sediments (Jürgens et al., 2002). To the best of our knowledge, no data are available for the

mineralization of EE2 in soil though it is suggested that the dissipation of EE2 in soil was in part microbially mediated (Colucci and Topp, 2001).

Several factors may affect biodegradation of steroid hormones in environment including temperature, redox conditions, organic matter content and soil moisture.

4.2.1. Temperature

In the loamy soils studied by Colucci et al. (2001a) soil temperature was found to influence the total mineralization of E2. Mineralization (61 days) decreased with decreasing temperature, with measured values being 14.7%, 14.1%, 10.9%, 6.0%, and 3.6% at 37, 30, 19, 10, and 4°C, respectively. Not surprisingly, the rates of degradation were slower at reduced temperatures, presumably due to reduced microbial activity.

4.2.2. Redox conditions

The former research suggests that redox conditions (aerobic, anoxic, or anaerobic) are critical factors for degradation of estrogens, as evidenced by batch experiments in agricultural top soil (Fan et al., 2007; Ying and Kaakana, 2005), river bed material (Jürgens et al., 2002), marine sediment (Ying and Kaakana, 2003) and aquifer material (Ying et al., 2003). The total mineralization of estrogens is generally higher under aerobic conditions than under anoxic/anaerobic conditions. Half-lives for the natural estrogens in aerobic sediment have been reported as <1 day but can be longer in anaerobic sediments with reports of up to 14 days for E1 (Jürgens et al., 2002). In transport studies with repacked soil columns (Das et al., 2004) and aerobic batch experiments with agricultural topsoils (Ying and Kaakana, 2005), both E2 and E1 dissipated in a similar manner. Mineralization of E1 in particular was reported to be highly affected by redox conditions (Fan et al., 2007; Ying and Kaakana, 2005). Under aerobic conditions E2 (applied as 2.5 mg/kg soil) was rapidly biotransformed into E1 with a DT_{50} (i.e., time to dissipate 50% of the initial concentration) of 3 days, and the generated E1 dissipated to an undetectable level of 0.1 mg/kg within 3 days. Under anaerobic conditions, however, E2 was transformed much more slowly (DT_{50} 20 days) and the generated E1 persisted in the soil without further dissipation throughout the 70-day experiment (Ying and Kaakana, 2005).

Similar results are obtained by Czajka and Londry (2006), whose tests were conducted under strict anaerobic conditions including sulphate, nitrate and iron reducing, and methanogenic conditions with limited degradation of E2. The authors found even slower degradation of E2 under nitrate reducing conditions, while anaerobic degradation of EE2 was not observed over a 3 year time period (Czajka and Londry, 2006). Similar results indicated that approximately 50% of E2 was degraded after 70 d anaerobic incubation, while little or no degradation of EE2 was observed under anaerobic conditions (Ying and Kookana, 2003). Mashtare et al. (2013) reported that under nitrate- and sulfate-reducing conditions, degradation followed similar trends in half-lives, E2 < EE2 < E1, with degradation much slower under sulfate-reducing conditions. Fan et al. (2007) reported that after 132 h of incubation, 6% of 375 μg/kg ^{14}C-E2 could be mineralized to $^{14}CO_2$ in native soils under aerobic conditions while there were no $^{14}CH_4$ or other ^{14}C-labeled volatile organic compounds detected under anaerobic conditions. The growth of microbes might be limited due to the lower level of electron acceptor under anaerobic conditions thus reducing the metabolism of these hormones. Considering that wet soils, swamps, fresh water or marine sediments and aquifers are frequently oxygen limited, sub-surface soil environments where anaerobic conditions often occur can be a potential source for steroid hormones.

4.2.3. Organic matter content

It is worth noting that neither low organic content nor high organic content is favorable to the degradation of estrogens. For instance, the half-lives of E2 in a loam soil with 3.2% organic matter and a silt loam soil with 2.9% organic matter were 61 and 72 h at room temperature (Jacobsen et al., 2005). According to Herman and Mills (2003), addition of 1 mg/L dissolved organic carbon increased the biotransformation of E2. The observed increase of degradation could be attributed to an enhanced microbial growth in the soils caused by the rise of organic matter concentrations, since the estrogens themselves as sole source of carbon and energy seem too low in concentration to support extensive microbial growth. In the case of carbon-deficient soils, therefore, small quantity of additional organic matter may enhance biodegradation of trace organic contaminants.

Unfortunately, the simultaneous monitor of the characteristics of microbial communities was often ignored in such studies. On the other hand, Herman and Mills (2003) also discovered that high concentrations of readily available carbon sources inhibited E2 degradation. This suggests that the availability of higher concentration of readily biodegradable organic carbon in water, as well as in soil or sediment may negatively affect the degradation of E2. This could be explained by that elevated levels of organic content decreased the extractability and bioavailability of estrogens, which have certainly a tendency to partition into organic matter due to their moderate lipophilicity. Nonetheless, this explanation comes to existence based on the assumption of degradation only proceeding in the liquid phase. In fact, Das et al. (2004) did indicate that strong sorption of E2 did not hinder degradation and they hypothesized that E2 either can be degraded while sorbed or rapidly desorbed from the solid phase and then degraded in the liquid phase. Systematic research addressing the impact of organic matter on biodegradation of estrogens is highly recommended. More importantly, it is necessary to distinguish the difference between the effect of dissolved and particulate organic matter.

4.2.4. Soil moisture

With regard to soil moisture, its effect on estrogen degradation parallels the effect of organic matter content. That is, neither very low nor very high soil moisture is beneficial to estrogen degradation. Colucci et al. (2001a) indicated that the mineralization of E2 increased from <1% to 20% after 73 d of incubation when the moisture content of the sandy loam soil was increased from air-dry to 15%. However, when moisture content of the same soil was increased to field capacity (24%), the amount of E2 mineralized decreased sharply to 8%. Note that soil moistened to field capacity limits oxygen supply to aerobic biodegradation. This suggests that the persistence of steroid hormones is more likely in dried sandy soil, moistened soil at field capacity or saturated soils, which is mainly due to the fact that appropriate moisture is indispensable to normal physiological functioning of living microorganisms while water-saturated condition limits oxygen supply to aerobic biodegradation.

In addition, natural and synthetic steroids are generally dispersed into the soil environment within a complex matrix of solid and soluble organic compounds due to the agricultural use of municipal sewage sludge (biosolids) and animal manures as fertilizer. These organic matrices, rich in nutrients, organic matter, and microorganisms, will profoundly change soil conditions following application, and therefore could influence the degradation of estrogens. A few studies have therefore been addressed about the impacts of these matrices on the behavior of the steroids in soils. Jacobsen et al. (2005) conducted various laboratory studies to investigate the influence of various organic matrices (e.g., swine manure slurry and municipal biosolids) on the aerobic degradation of E2 in three agricultural soils the same as that used formerly by Colucci et al. (2001a). Their results indicate that the municipal biosolids, and to a lesser degree manure slurry accelerated the mineralization of [^{14}C]-E2 detected after 96 h of incubation. These results also indicate that microorganisms carried in manure can convert E2 to E1, and that mineralization of E2 requires a viable soil microbial population. Lucas and Jones (2006) similarly reported that the mineralization of E2 and E1 in the sheep manure amended soils was generally higher than in the non-amended soils. Additionally, in comparison to urine amended soil, the rate of estrogens mineralization is higher in cattle and sheep manure, which is also significantly affected by the age of the manures. It is well known that sorption of organic compounds increases with the organic contents of the soil.

The addition of manure or biosolids may, therefore, enhance the sorption of estrogens due to the combination of elevated levels of organic carbon content and higher adsorption surface area in soil. However, strong sorption of parent compound and transformation products was shown not to hinder degradation, as the high rate of degradation could only be reached if compounds were either degraded while sorbed or rapidly desorbed from the solid phase and then degraded in the aqueous phase (Das et al., 2004). Two possible hypotheses explain the elevated degradation: Firstly, the introduction of the microorganisms inherent to organic matrices that are both adapted to the chemical conditions and possess the intrinsic capability for estrogen degradation; also, the microbial activity in the soil is stimulated due to the nutrient addition introduced by application of organic matrices.

The former presumption seems to be more rational (Das et al., 2004; Lucas and Jones, 2006). In the case of agricultural sites with different long-term treatment histories with wastewater and sewage sludge, however, Stumpe and Marschner (2007) reported that the long-term sewage sludge application had no effect on E2 mineralization whereas long-term wastewater irrigation significantly decreased E2 mineralization rate in soils compared to the corresponding soil with freshwater irrigation. Further work is needed to elucidate the discrepancy between the long-term and short-term effect of organic matrices application on the degradation of estrogens in soils. As a whole, given the complex nature of real-world situations where soil pH, redox conditions, temperature, prevailing soil water conditions, wetting and drying cycles, as well as the fact that the size and type of bacterial populations can vary, biodegradability of estrogens may likely to be different in the field than what has been observed under controlled conditions in the laboratory.

The degradation pathway, conversion of E2 to E1, and subsequent formation of non-extractable residues was observed consistent with three kinds of soils under all incubation conditions (Colucci et al., 2001a). A miscible-displacement column experiment indicated that E2 entered the soil column underwent rapid transformation to form at least three metabolites, E1, sporadic E3 and an unidentified high-polarity compound (Casey et al., 2003). Results from batch incubation experiment also indicated that E2 could be transformed to an unidentified polar compound through abiotic chemical processes in agriculture soil (Fan et al., 2007). Unfortunately, all of these studies failed to identify the unknown intermediate degradation products.

The authors concluded that estrogens are biodegradable in soils by ubiquitous microorganisms that require no prior adaptation (Colucci et al., 2001b). Stumpe and Marschner (2007) also indicated that two-week pre-incubation of the soils with 0.1 mg/kg unlabeled hormones or application of the hormones within a wastewater matrix had only minor effects on their mineralization.

While estrogens were studied to be biodegraded in soil, little was known about the soil microorganisms responsible for estrogen degradation. The first attempts to identify the microorganisms involved in estrogens biotic transformation, over 60 years ago, were based on culture-dependent techniques (Turfitt et al., 1947).

In this study, the biodegradation of E1 was examined using 355 different cultures of bacteria

isolated from five disparate soil types. No culturable bacteria were found in loam, marl, or alkaline peat soils that could metabolize E2. However, one *Proactinomyces spp*. was isolated from an acid sand, and two strains were found in arable soil that could use E2 as a carbon source. E1 was degradable by one species of *Proactinomyces spp*. in the arable soil, but no degradation was observed with organisms from the other four soils.

4.3. Comparison of degradation rates in the natural system

Half-lives of each estrogens in different natural systems vary. Research conducted by Moschet (2009) and Xuan et al. (2008) suggested that half-lives of E1 and E2 in aerobic water are pretty close, while degradation rate of E2 is significantly higher than that of E1 in aerobic soil and sediment. Generally, EE2 is harder to be degraded as compared to E2 and E1, as the ethinyl group makes it more recalcitrant (Colucci and Topp, 2001; Colucci et al., 2001b; Jürgens et al., 2002; Ying and Kookana, 2003).

On the other hand, degradation rate under anaerobic conditions is much lower than under aerobic conditions or sometimes degradation cannot even proceed, as evidenced by Ying and Kookana (2003) and Czajka and Londry (2006). Degradation of E2 in the anaerobic river sediment is much faster than that in the lake sediment and anaerobic marine, may due to that there was still a tiny amount of oxygen in the anaerobic river sediment because it is difficult to create a real anaerobic environment (Moschet, 2009).

4.4. Degradation in manure

Large estrogen loads associated with animal feeding operations (AFOs) arise not only from steroidal treatments given to livestock to enhance growth and fecundity, but also from the current trend towards concentrating animals into small geographic areas, magnifying waste related issues. Manure disposal presents a potential source for surface and groundwater contamination, consequently threatening human health and imperiling wildlife communities.

Bacteria in animal manure are capable of degrading estrogens. Soils amended with swine manure facilitate the biodegradation of estrogens, mainly due to the presence of fecal bacteria. Raman et al. (2001, 2004) tested estrogen degradation in cattle and swine manures. The authors found that the E2 concentration dropped sharply during the first 24 h of incubation under aerobic conditions; while E1 was accumulated and reached a peak concentration in 48 h. The total estrogenic activity measured by yeast screen decayed following first order kinetics, and the rate constants increased with temperature from 0.03 day^{-1} at 3°C to 0.12 day^{-1} at 5°C. The manure microorganism *Cornybacterium* spp. was believed to be responsible for the biodegradation of both E1 and E2.The complete biodegradation of estrogen compounds is largely dependent on the destruction of the phenolic ring. The fungus group *Paecilomyces lilacinus* was found to be capable of cleaving the phenolic ring of biphenyl into five di- and trihydroxylated metabolites and thus was believed to have the capacity to degrade estrogen compounds as well (Gesell et al., 2001). *Fusarium proliferatum* isolated from a manure sample, could degrade EE2 with an initial concentration of 25 mg l^{-1} (Shi et al., 2002).

The impacts of pH values, temperature, the presence of antibiotics, and incubation time on degradation efficiency of estrogens in manure have been observed in many studies. Shore et al. (1993) incubated broiler litter for 1 week at different pH values, with and without the addition of antibiotics (penicillin/streptomycin), and found significant reductions in estrogen concentrations at pH 5 and 7 but no change at pH 1 or 12.

When antibiotics were added to the litter, estrogens persisted. Similar results indicated that estrogens may have slower dissipation rates in soil particularly when manure is applied at high rates or not mechanically mixed in the surface horizon (Rose and Farenhorst, 2014). Tetracycline in manure induced a lag phase of 40 to 50 days, and tetracycline at 200 mg kg^{-1} remarkably decreased maximum mineralization of E1 and E2 in manure. Schlenker et al. (1998) studied the degradation of estrogens in cattle feces by incubating manure samples for 12 weeks at 20-23°C. The median concentrations of total estrogens extracted from the manure were unchanged for 9 weeks but were reduced by 80% after 12 weeks. Schlenker et al. (1999b) tested *E. coli* and *Clostridium perfringens* for their ability to degrade fecal estrone in cattle manure. The *E. coli* had no effect on estrone concentrations, but the *C. perfringens* reduced the average concentration of estrone from ~16 μg L^{-1} to ~11 μg L^{-1} during the 48 h incubation. Schlenker et al. (1999a) evaluated the influence of temperature on the stability of estrogens in the feces of cattle. At 5°C, the median concentrations of total estrogens extracted from the manure fell below initial concentrations after 12 weeks of incubation. At 30 °C, however, estrogen was almost completely eliminated from the samples within 3 weeks. Similar studies of estrogen degradation in dairy cattle manure were done by Raman et al. (2001). Press cake samples were spiked with 17β-estradiol and incubated at temperatures ranging from 5 to 50°C. The effects of acidification on estrogen transformation and degradation during sample storage were also evaluated. At all temperatures, estradiol concentrations rapidly declined during the first 24 h of incubation, and estrone accumulated. Total estrogen removal rates followed the pattern of estrone degradation, and these data were fitted to a first-order decay model. Rate constants increased from ~0.03 d^{-1} at 5°C to ~0.12 d^{-1} at 50°C. Acidification to pH 2 reduced rates of estrogen transformations at both 5 °C and 30°C, but a 15% and 31% loss, respectively, of total estrogen was still observed when samples were stored for 7 days. The authors speculated that *Cornybacterium spp.* were

partially responsible for the estrogen transformations in their study (2001). Hakk et al. (2005) performed incubation experiments under aerobic conditions to assess the water extractability of E2 in chicken manure compost with 60% moisture. They concluded that the extractability of E2 decreased with time, and the first-order degradation rate constant for E2 was 0.010 d^{-1}, and the first-order degradation rate constant for E2 was 0.010 d^{-1}. Chun et al. (2005) suggested that the presence of antibiotics significantly decreased transformation of E2 to E1 upon recommendation that any study evaluating the fate and transport of estrogenic hormones in soil should include the effect of agricultural antibiotics because antibiotics and estrogenic hormones were commonly excreted together in environmental samples.

4.5. Degradation in biological wastewater treatment systems

4.5.1. Field monitor data

Different studies around the world agree on the aerobic degradation of estrogens underwent during aerobic biological treatment units in municipal STPs. Municipal STP is an important facility which markedly reduces the concentrations of estrogens, although its principal purposes are the removal of organic substances even nitrogen and phosphorus from wastewater, as well as the disinfection of the treated wastewater. There is abundant documentation about the overall removal efficiencies of estrogens, as determined from influent and effluent concentrations in actual STPs situated in Europe, North America, Australia and Japan. It was proved to be efficient in removing E2 and E3, but less effective in removing E1 and EE2 (Baronti et al., 2000), similar to the findings by Shi et al. (2004a). Moreover, this conclusion was confirmed by the experiment using two pilot-scale STPs free from the problems such as fluctuations in influent concentrations, cleavage of conjugated hormones, rainfall events, which were inevitably encountered when dealing with full-scale plants (Esperanza et al., 2004). In anaerobic condition, estrogens removals were in the range of 76-92% for E1, 58-90% for E2, 43-63% for E3, and 62-88% for EE2. In aerobic phase, removal of estrogens were ranging from 79-96% for E1, 76-96% for E2, 36-64% for E3, and 57-96% of EE2 (Ruchiraset and Chinwetkitvanich, 2014). To our best knowledge, among all of the published field monitor data all over the world, the Wiesbaden STP, a common municipal STP in German, with an activated sludge system for nitrification and denitrification including sludge recirculation, operated with a sludge retention time (SRT) of 11-13 d, had the best removal rate not only for E1 and E2, but also for EE2, which was more than 99%, 98% and 93% respectively (Andersen et al., 2003).

With the attempt to evaluate the importance of sorption and biodegradation, which are widely viewed as two principal mechanisms for estrogen removal by biological treatment, more detailed studies also have been conducted on the fate of estrogens in each treatment step of specific STPs. A complete mass balance of Wiesbaden STP based on concentration profiles in both water and sludge showed that biodegradation was the main pathway for estrogens removal, which was responsible for above 80% removal for both natural and synthetic estrogens, whereas only approximately 5% of the estrogens entered the plant were removed with excess sludge (Andersen et al., 2003). Another study case of Danish Lundtofte STP suggested that removal of estrogens with excess sludge was less than 2% of the total amounts of estrogens that entered the STP (Andersen et al., 2005). However, in a sewage treatment plant in Centre Eastern Tunisia using simple activated sludge process, sorption onto sludge played a predominant role in the removal of estrogens in warm season, especially for E1 and E2 (69.5 and 66.3%, respectively) (Belhaj et al., 2013). This conclusion was evidenced by Xu et al. (2014), Zhang et al. (2014), Zeng et al. (2013) and Ruchiraset and Chinwetkitvanich (2014). So it is noteworthy that sorption may affect the fate of estrogens in STPs because estrogens are easily sorbed onto activated sludge (Zeng et al., 2013).

4.5.2. Lab-scale batch tests of sludge culture

Aerobic degradation

To observe the biodegradation of estrogens under different conditions, extensive research has been performed with real activated sludge from various STPs. The efficiency of a STP to degrade estrogens is influenced by numerous parameters including microbial activity, sludge retention time (SRT) (Maeng et al., 2013), hydraulic retention time (HRT), temperature, and rainfall, all of which vary seasonally (Ternes, 1998). For example, SRT was proved to be a suitable operational parameter to remove estrogens in activated sludge system. Zeng et al. (2013) investigated the effect of SRT on the removal and fate of E2 and EE2 in an anaerobic–anoxic–oxic activated (A^2/O) sludge system by lab-scale experiments. Optimal SRT was 20 days for E2, EE2 and nutrient removal. Mass balance calculation suggested that 99% of influent E2 was degraded by the activated sludge process, and 1% remained in excess sludge; of influent EE2, 62.0%–80.1% was biodegraded; 18.9%–34.7% was released in effluent; and 0.88%–3.31% remained in excess sludge. Different treatment systems may also affect microbial activity and therefore, estrogenic composition of STP effluents (Rodgers-Gray et al., 2000). The conventional activated sludge (CAS) process has been reported to have higher estrogen removal efficiency than trickling filter (Chimchirian et al., 2007). Furthermore, it appears that oxidation ditch (OD) process is better than CAS process, while the biological nitrogen removal process with high SRT removes natural estrogens as effectively as the OD plants (Hashimoto et al., 2007).

In batch experiments, Ternes et al. (1999b) investigated the basic aerobic microbial reactions of

estrogens in activated sludge taken from the old Wiesbaden plant with only BOD removal. Experiments were performed at 20°C with two starting concentrations of 1 μg/L and 1 mg/L for E2. At both spiking levels, E2 was almost quantitatively oxidized to E1. It was also found that the removal of E2, as well as the formation and elimination of E1 was accelerated at the lower spiking level, under which condition neither E2 nor E1 was to be found above the detection limits after 5h. Layton et al. (2000) conducted a similar study with estrogens using activated sludge from four municipal STPs in Tennessee U.S.A. The sludge from all of the municipal plants mineralized 70-80% of radio labelled ^{14}C-E2 to ^{14}C-CO_2 within 24 h. A recombinant yeast estrogen assay (YES assay) also confirmed that biological estrogenic activity was removed from the biosolid samples to below the detection limit (1.56 nM) indicating no accumulation of E1. In this study, ^{14}C EE2 was found to metabolize much more slowly, even though 40% was mineralized in 24 h. The degradation activity of E1 and E2 is seen to be higher in the sludge from membrane bioreactor (MBR) plant than that from conventional activity sludge (CAS) plant by a factor of 2-3, which was attributable to either higher age of the sludge (30 d vs 11 d) resulting higher accumulation of specialist-degrading estrogens, or smaller sludge flocs size (80 vs 400 μm) with thinner boundary layer favoring the passive-diffusion mass transfer (Joss et al., 2004).

Essandoh et al. (2012) studied the removal of three potent EDCs including estrone (E1), 17β-estradiol (E2), and 17α -ethinylestradiol (EE2) in a wastewater using soil columns. E2 was the most easily removed estrogen, while EE2 was the least removed. Besides, novel bioreators are efficient in removal of estrogens, such as membrane distillation bioreactor (MDBR) which can be operated at thermophilic conditions to facilitate the integration of biological treatment with membrane distillation (Wijekoon et al., 2014). In order to know the more exact behavior of estrogens in actual sewage when in contact with activated sludge, Suzuki and Maruyama (2006) conducted an experiment using filtered sewage and activated sludge that were both collected from STPs in Japan. E2 and E1 simultaneously decreased immediately after the beginning of the batch mixing. E2 rapidly decomposed completely within 4-6h, while E1 concentration remained constant at 2-5 ng/L following the incipient rapid descent during a period of 24h. The biodegradation in the sludge phase was assumed by many researchers following a pseudo-first-order reaction as given below (Eq. 1):

$$dC/dt = K \cdot TSS \cdot C \quad (1)$$

where K is the first-order biological degradation rate constant, in L/(g of TSS·d); TSS is the mixed liquor suspended solids, in g/L; and t is the time of degradation in sludge, in day.

The integration of Eq. (1) will result in:

$$C_t = C_0 \cdot \exp(-K \cdot TSS \cdot t) \quad (2)$$

where C_0 is the initial concentration and C_t is the concentration at time t (days), in ng/L.

All the K-values obtained directly or standardized from literature are summarized in Table 3. Despite that the K-values are markedly different, some conclusions can be generalized as following. Firstly, the sequence of K-values is E2>E1>>EE2 for the same sludge. Secondly, much lower were the initial estrogens concentrations; much higher K-values would be obtained, which indicate the sludge activity could be inhibited by high concentrations estrogens. Fortunately, the estrogens concentrations of actual sewage never exceed 200 ng/L according to all of the published monitor data up to the present. It is expected that estrogens could be biotransformed at relatively high rates. However, these previous batch tests tended to overestimate the true degradation rates, which could be attributed to two reasons. On the one hand, the experiments were carried out with the estrogens solution lacking organic substrates that could competitively inhibit the degradation of estrogens (Joss et al., 2004). On the other hand, these aeration experiments were likely to be consistently high in a shaking flask, which may not always be the case within an activated sludge aeration tank.

Moreover, the occurrence of nitrification in an activated sludge system seems to have a positive effect on the removal of hormones. For nitrification a longer SRT is required because the autotrophic bacteria involved grow very slow (de Mes et al., 2005).

It is noteworthy that heterotrophic populations, which are likely to affect estrogen degradation, can be influenced by the type of growth substrate. Ziels et al. (2014) reported that kinetic rate coefficient (k_b) values for EE2 biodegradation ranged from 5.0 to 18.9 L/g VSS/d at temperatures of 18°C to 24°C. EE2 k_b values for aerobic biomass growth at low initial food to mass ratio feeding conditions (F/M_f) were 1.4 to 2.2 times greater than that from growth at high initial F/M_f, which indicated that employing MBR processes (no gravitational settling) with low-F/M_f growth conditions facilitated EE2 biodegradation. Similarly, oxidation ditch (OD) processes, which can provide low-F/M_f substrate removal conditions, might promote higher EE2 biodegradation kinetics, while keeping acceptable sludge settling characteristics.

Tan et al. (2013) pointed out that further enrichment under starvation conditions may enhance E1 degradation capability via the growth and/or stimulation of multiple substrate utilizers rather than heterotrophs which are characterized by an r-strategist growth regime, although initial growth of biomass depends on the presence of sufficient organic carbon. More research is needed to determine whether and how estrogen degradation is affected by F/M_f conditions.

Anoxic and anaerobic degradation

Anoxic is most commonly defined as the absence of oxygen, while *anaerobic* indicates the absence of a common electron acceptor such as nitrate, sulfate or oxygen.

Table 3. *K*-values obtained directly or standardized from literature

Initial concentration	*E2*	*E1*	*EE2*	*References*
100 ng/L			8	Joss et al., 2004
500 ng/L	350	162		Joss et al., 2004
1μg/L	150	20	0	Ternes et al., 1999a
50μg/L			0.6	Vader et al., 2000
58μg/L	2.79			Layton et al., 2000
72μg/L			0.13	Layton et al., 2000
200μg/L	*11.66*	*1.2*	*1.34*	Shi et al., 2004a
1.0 mg/L	*8.36*	*0.42*	*0.2*	Shi et al., 2004a
20-25mg/L	0.22	0.16	0	Shi et al., 2004b

Matsui et al. (2000) is probably the first to suggest that estrogen activity was mainly reduced during the denitrification treatment step which was also the first step of the activated sludge treatment by performing a detailed profile of estrogen removal in a Japanese STP using an immunoassay for E2 in combination with the yeast estrogen screening (YES) assay for measuring estrogen activity.Then Andersen et al. (2003) further demonstrated that estrogens were mainly degraded during denitrification based on analysis of steroid estrogens in both water and sludge in a south German STP by GC-MS-MS. Afterwards, Joss et al. (2004) systematically investigated the degradation of estrogens under different redox conditions in batch experiments performed with same activated sludge originating from either CAS or MBR. The degradation rate of E1 and EE2 depended strongly on redox conditions, while E2 exhibited high rates anyway. For example, the *K*-value ($L \cdot gSS^{-1} \cdot d^{-1}$) of E1 degraded by CAS sludge was 10 under anaerobic conditions, 30 under anoxic and 162 under aerobic conditions (Joss et al., 2004). With regard to E2, the K-value was 350 and 460 with CAS sludge under aerobic and anoxic conditions respectively, while was 950 and 500 with MBR sludge under aerobic and anaerobic conditions respectively. In contrast, Dytczak et al. (2008) indicated that the conversion from E2 to E1 was faster under aerobic than anoxic conditions and removal of E1 was not observed over the 3-7 hour incubation times. The influence of oxidation-reduction conditions on E2 degradation currently remains ambiguous. The exiting data implied that EE2 was degraded under neither anoxic nor anaerobic conditions (Czajka and Londry, 2006; Dytczak et al., 2008; Joss et al., 2004).

A denitrifying bacterium was lately isolated from activated sludge of a municipal wastewater treatment plant using E2 as sole source of carbon and energy (Fahrbach et al., 2006).

Estrone was converted to 17α-estradiol under either nitrate-reducing condition (Dytczak et al., 2008) or anaerobic condition (Czajka and Londry, 2006). In some respects this could be considered as a deactivation step, in that 17α-estradiol is less biologically active than E2 (Hutchins et al., 2007). However, it is not known to what extent 17α-estradiol remains stable in anaerobic/anoxic conditions, and the potential therefore exists for reverse transformation back to E1 and ultimately back to E2.

However, little research has been conducted on the microbial degradation of estrogens under anaerobic conditions. Overall anaerobic conditions resulted in much slower conversion rates compared to the same experiments under aerobic conditions. For example, a half-life of 2.5 min for E1 under aerobic conditions, was 1.66 h under anaerobic conditions. No degradation of the three estrogens was found by Pakert et al. (2003) in batch experiments using sludge from anaerobic digester. Zhang et al. (2013) reported that the degradation rate constant of estrogens decreased with the original estrogen concentrations while increased with the sludge concentrations. Additionally, E1 was accumulated in the process of E2 degradation. The degradation rate of E1 decreased by a factor of between 3 and 5 in the transition from aerobic (O_2 available in solution) to anoxic (nitrate available but no molecular oxygen) as well as from anoxic to anaerobic (Joss et al., 2004).

Bed sediment was used to examine the potential for E2 to be degraded anaerobically at 20°C; and was fairly rapidly converted to E1, almost completely after an incubation of 2-days (Jürgens et al., 2002). In batch experiments with activated sludge supernatant under anaerobic conditions (purged with nitrogen gas), after 7 days, 50% of the spiked amount of E2 was converted into E1 and resulting in accumulation of E1 due to no further degradation (Lee and Liu, 2002).

EE2 tested under anaerobic conditions in river water samples showed no degradation over 46 days (Jürgens et al., 1999). Under strict anaerobic conditions, E1 is expected to convert into E2, rather than E2 is converted to E1 (Joss et al., 2004). So somehow under anaerobic conditions there are still electron acceptors available, like Fe^{3+} and various organic oxidative compounds, responsible for the conversion. Joss et al. (2004) also found the conversion of EE2 in MBR sludge under anaerobic conditions was nearly the same as that in the blank experiment where no sludge was present.

On the whole, the microbial conversion rates of estrogens under various oxidation-reduction conditions appeared to follow the order: aerobic > anoxic > anaerobic.

5. Metabolic pathways

5.1. Isolated organisms and enzymes involved in estrogens degradation

Several reports dealing with isolating and identifying microorganisms are involved in estrogens degradation. For instance, Yu et al. (2005) indicated that a frequent occurrence of *E.coli*, *Pseudomonas fluorescens* (gram negative), and *Bacillus thuringiensis* (gram positive) strains were probably responsible for the estrogen-degrading capacity in activated sludge by using the method of terminal restriction fragment length polymorphism (T-RFLP) assay. The bacterial strains that are capable of degrading estrogens are briefly summarized below in Table 4. A strain of the gram-negative, oval-shaped aerobic bacterium genus *Novosphingobium tardaugens* sp. nov. isolated from activated sludge decomposes E1, E2, E3, except for EE2. In addition, this bacterium seemed to assimilate E2 as a preferred carbon source and the degradation activity was not accelerated by yeast extract or glucose (Fujii et al., 2002; Fujii et al., 2003), which was similar to what was observed in *Rhodococcus zopfii* (Yoshimoto et al., 2004).

Although bioreactors at a wastewater treatment plant were operated under eutrophic conditions, it was suggested that these bacterial strains possibly degrade estrogens selectively in such environment. Similarly, two another gram-negative bacilus, *Achromobacter xylosoxidans* and *Ralstonia picketii*, isolated from membrane bioreactor (MBR), were able only to degrade E1, E2, E3, but not EE2 (Weber et al., 2005).

Table 4. Microorganisms capable of biodegrading or metabolizing natural and synthetic estrogens

Resources	***Strains***	***Generas***	***Gram Stain***	***E1***	***E2***	***E3***	***EE2***	***References***
Sewage treatment plants	*Novosphingobium tardaugens* sp. nov.	*Novosphingobium*	negative	○[a]	○	○	×[b]	Fujii et al., 2002, 2003
	Rhodococcus zopfii	*Rhodococcus*	-[c]	○	○	○	○	Yoshimoto et al., 2004
	Rhodococcus equi	*Rhodococcus*	-	○	○	○	○	Yoshimoto et al., 2004
	EMS-1	*Rhodococcus*	-	○	○	○	-	Villemur et al., 2013
	JEM-1	*Novosphingobium*	negative	○	○	○	○	Hashimoto et al., 2009
	Achromobacter xylosoxidans	*Achromobacter*	negative	○	○	○	×	Weber et al., 2005
	Ralstonia picketii	*Ralstonia*	negative	○	○	○	×	Weber et al., 2005
	E2Y1	*Bacillus*	-	○	○	-[d]	-	Jiang et al., 2010
	E2Y2	*Bacillus*	-	×	○	-	-	Jiang et al., 2010
	E2Y3	*Bacillus*	-	×	○	-	-	Jiang et al., 2010
	E2Y4	*Bacillus*	-	○	○	-	-	Jiang et al., 2010
	E2Y5	*Bacillus*	-	×	○	-	-	Jiang et al., 2010
	KC6	*Aminobacter*	negative	○	○	-	-	Yu et al., 2007
	KC7	*Aminobacter*	negative	○	○	-	-	Yu et al., 2007
	KC12	*Brevundimonas*	negative	×	○	-	-	Yu et al., 2007
	KC13	*Escherichia*	negative	×	○	-	-	Yu et al., 2007
	KC1	*Flavobacterium*	negative	×	○	-	-	Yu et al., 2007
	KC2	*Flavobacterium*	negative	×	○	-	-	Yu et al., 2007
	KC5	*Microbacterium*	positive	×	○	-	-	Yu et al.,2007
	KC3	*Nocardioides*	positive	×	○	-	-	Yu et al., 2007
	KC4	*Rhodococcus*	positive	×	○	-	-	Yu et al., 2007
	KC8	*Sphingomonas*	negative	○	○	-	-	Roh and Chu, 2010; Yu et al., 2007
	KC9-11	*Sphingomonas*	negative	×	○	-	-	Yu et al., 2007
	KC14	*Sphingomonas*	negative	×	○	-	-	Yu et al., 2007
	Nitrosomonas europaea	*Nitrosomonas*	negative	○	○	○	○	Shi et al., 2004 Yi et al., 2007
Soil samples from agricultural fields	ED8	*Sphingomonas*	negative	○	○	-	-	Kurisu et al., 2010
	ED9	*Sphingomonas*	negative	○	○	-	-	Kurisu et al., 2010
	ED7	*Rhodococcus*	positive	○	○	-	-	Kurisu et al., 2010
	ED10	*Rhodococcus*	positive	○	○	-	-	Kurisu et al., 2010
Marine sand and seawater	CYH	*Sphingomonas*	negative	○	○	×	×	Ke et al., 2007
	LHJ1	*Acinetobacter*	negative	×	○	×	×	Ke et al., 2007
	LHJ3	*Agromyces*	positive	×	○	○	×	Ke et al., 2007
	S19-1	*Buttiauxella*	negative	-	○	-	-	Zhang et al., 2011
	H5	*Vibrio*	negative	-	○	-	-	Sang et al., 2012

Wastewater treatment facility of contraceptive factory	*Sphingobacterium sp.* JCR5	*Sphingobacterium*	negative	○	○	○	○	Haiyan et al., 2007
Manure of cowshed compost	*Fusarium proliferatum* strain HNS-1	*Fusarium*	-	-	-	-	○	Shi et al., 2002
	Phyllobacterium myrsinacearum (BP1)	α-Proteobacteria	negative	○	○	○	○	Pauwels et al., 2008
	Ralstonia pickettii(BP2)	β-Proteobacteria	negative	○	○	○	○	Pauwels et al., 2008
	Pseudomonas aeruginosa(BP3)	γ-Proteobacteria	negative	○	○	○	○	Pauwels et al., 2008
	Pseudomonas sp. (BP7)	γ-Proteobacteria	negative	○	○	○	○	Pauwels et al., 2008
	Acinetobacter sp. (BP8)	γ-Proteobacteria	negative	○	○	○	○	Pauwels et al., 2008
	Acinetobacter sp. (BP10)	γ-Proteobacteria	negative	○	○	○	○	Pauwels et al., 2008

a: able to degrade the corresponding pollutant; b: disable to degrade the corresponding pollutant; c: unsuitable for Gram stain method or undetected; d: undetected

More recently, fourteen phylogenetically diverse E2-degrading bacteria, which widely distributed among eight different generas *Aminobacter*, *Brevundimonas*, *Escherichia*, *Flavobacterium*, *Microbacterium*, *Nocardioides*, *Rhodococcus*, and *Sphingomonas* of three Phyla: Proteobacteria, Actinobacteria, and Bacteroidetes, were successfully isolated from activated sludge of a wastewater treatment plant (Yu et al., 2007).

So far, only a few strains, like *Rhodococcus zopfii* and *Rhodococcus equi*, isolated from activated sludge were found to degrade not only natural estrogens but also the synthetic estrogen EE2 (Yoshimoto et al., 2004). The rare presence of EE2-degrader in activated sludge can be applied to account in part for the frequent observation of inefficient removal in real-scale wastewater treatment plants and slow mineralization in batch experiments upon EE2. Larcher and Yargeau (2013) found that *R. rhodochrous* was able to degrade EE2 with no detectable EE2 after 48h, and that no additive or synergistic effects were observed due to the combination of the bacterial cultures with maximum EE2 removals of 43% after 300h. The two *Rhodococcus* strains degraded E2 and E1 by 99% or more, as well as degraded E3 and EE2 by 80% or more in 24h with an initial concentration of 100 mgL^{-1} (Yoshimoto et al., 2004). In addition, *Rhodococcus zopfii*, one of the two *Rhodococcus* strains, showed particularly strong degrading activities, which degraded E2 (100 mg/L, 10 ml) by 81% in 2 h, E1 at the same concentration by 91% in 3 h, E3 by 96% in 4h, and EE2 by 70% in 6 h (Yoshimoto et al., 2004). *Rhodococcus* sp. strains have been reported to degrade cholesterol that possesses a steroidal skeleton and to degrade aromatic compounds such as 2,4-dinitrophenol, polychlorinated biphenyl (Maeda et al., 1995; Yazdi et al., 2001; Yoon et al., 2000). *Achromobacter* sp. and *Ralstonia* sp. are known to be able to degrade some pollutants, especially phenolic compounds (Bhushan et al., 2000; Shin et al., 2003). *Novosphingobium*, which is one of the recently proposed genera which were previously included in the genus *Sphingomonas* (Takeuchi et al., 2001), is also well known for including many species that can assimilate biodegradation-resistant compounds such as chlorophenol (Nohynek et al., 1996), fluorene, biphenyl, dibenzothiophene (Frederickson et al., 1995), benzoate, cresol, naphthalene and xylene (Balkwill et al., 1997). Thus, it might be inferred that the known strains being able to degrade compound carrying phenolic group also could assimilate estrogens. It has been proved by Haiyan et al. (2007) that *Sphingobacterium* sp. JCR5 which grew on EE2 as sole source of carbon and energy could also degrade E1 and E2.

Besides activated sludge, some estrogen-degrading bacteria were also successfully isolated from soil, manure and compost. The first attempts to identify the microorganisms involved in estrogens biotic transformation in soil, over 60 years ago, were based on culture-dependent techniques (Turfitt et al., 1947). In this study, the biodegradation of E1 was examined using 355 different cultures of bacteria isolated from five disparate soil types. No culturable bacteria were found in loam, marl, or alkaline peat soils that could metabolize E2. However, one *Proactinomyces spp*. was isolated from an acid sand, and two strains were found in arable soil that could use E2 as a carbon source. E1 was degradable by one species of *Proactinomyces spp*. in the arable soil, but no degradation was observed with organisms from the other four soils. Three estrogen-degrading bacteria, LHJ1, LHJ3, and CYH, were isolated from microcosms constructed with marine sand and ultrafiltered (UF) secondary effluent (Ke et al., 2007). All three isolates were not capable of degrading EE2. Under aerobic condition, all three isolates oxidize E2 to E1, while only one of them, CYH, degraded E1, and only LHJ3 degraded E3. Furthermore, under aerobic condition, CYH was able to degrade E1 while LHJ3 was able to degrade E2.

Five species, i.e. *Phyllobacterium myrsinacearum*, *Ralstonia pickettii*, *Pseudomonas aeruginosa*, *Pseudomonas* sp. and *Acinetobacter* sp. isolated from compost were able to degrade E1, E2 and E3, while were not able to degrade EE2 except in the presence of E2 (Pauwels et al., 2008).

Moreover, fungi were found to be able to degrade estrogens as well. For example, an EE2-

degrading fungus (*Fusarium proliferatum*) was isolated from cowshed samples (Shi et al., 2002). Ligninolytic fungi (LF) or their enzymes can be actively used for decontamination because of their ability to either polymerize the target pollutants or to substantially decompose the original structure using ligninolytic enzymes and cytochrome P-450 (Cajthaml, 2015). Likewise, white-rot fungi (WRF) and their lignin modifying enzymes (LME) can degrade various kinds of trace organic contaminants (TrOC). Depending on the initial concentration of TrOC, WRF species and associated LME, addition of redox mediators can facilitate the removal of those compounds (Yang et al., 2013). *T. versicolor* was also able to degrade EE2 (Castellana and Loffredo, 2014). Many studies were also performed in order to determine the feasibility of applying fungal treatments to the biodegradation of estrogens, as shown in Table 5. In light of the recent success in the isolation of estrogen-degrading cultures from activated sludge and different environmental compartments, natural estrogen-degrading cultures are widespread in environment and engineered systems. Natural estrogens of human and animal origin are being delivered into the environment over thousands of years, especially due to growing population and more intensive farming.

It is therefore not surprising that a large number of bacteria have acquired pathways to make use of these compounds as growth substrates. Certain bacteria are able to grow on steroid hormones as the sole source of carbon and energy by the expression of a set of steroid-catabolizing enzymes. By contrast, so far only a small number of EE2 degrading microbes have been isolated. However, it remains uncertain whether the different degradation abilities of estrogen-degrading cultures toward estrogens contribute to the wide range of estrogen removal observed by many field studies.

Another important issue is to know whether these isolated strains are still active in the case estrogens at the environmentally relevant concentration, due to the fact that estrogens were generally found in natural environments in nanograms-per-liter concentrations.

5.2. Degradation pathways of E1 and E2

Although bacteria are generally capable of growing on either natural or synthetic estrogens as sole source of carbon and energy, only a few metabolic pathways have been proposed. Bolton et al. (1998) suggested a metabolic pathway where E2 is oxidized to E1 and then further oxidized to a more polar semiquinone or quinone. Only a small fraction of E3 is found in effluent probably due to the three hydroxyl groups in its structure, which makes it more susceptible of surface reactions.

E1 might be one of the common key intermediates involved in the degradation of E2, which was confirmed by both laboratory studies performed with either activated sludge or soil (Colucci et al., 2001a; Jacobsen et al., 2005; Johnson et al., 2001; Lee et al., 2002; Shi et al., 2013; Ternes et al., 1999a; Weber et al., 2005; Yu et al., 2007) and field monitor data (Baronti et al., 2000). In a pilot-scale step-feed anoxic/oxic (A/O) wastewater treatment system established by Shi et al. (2013), a linear relationship between the concentrations of E1 and E2 in the water phase indicated the transformation between E1 and E2. The equilibrium constant (K) for the transformation between E1 and E2 was remarkably higher in the anoxic zones (0.38–0.81) than in the aerobic zones (0.08–0.24). The frequently observed conversion of E2 to E1 is probably due to microbial enzymatic activity of an NADH-dependent hydroxysteroid dehydrogenase (Czajka and Londry, 2006). A miscible-displacement column experiment indicated that E2 entered the soil column underwent rapid transformation to form at least three metabolites, E1, sporadic E3 and an unidentified high-polarity compound (Casey et al., 2003). It is worth noting that, E2 also can be biotransformed in some other pathways not via E1. For instance, Degradation of E2 by neither *Rhodococcus zopfii* (Yoshimoto et al., 2004) nor commercial pure culture *Nitrosomonas europaea* (Shi et al., 2004) occurred with increasing of E1 concentration. In another study, a new metabolite, a steroid-D-ring lactone, was detected during the very early stages of E2 degradation by sewage bacteria (Lee and Liu, 2002).

Table 5. Fungal enzymes capable of biodegrading or metabolizing estrogens

Treatment	*Fungal enzymes*	*Initial concentration*	*E1 removal rates*	*E2 removal rates*	*E3 removal rates*	*EE2 removal rates*	*References*
Flask scale	VP from *B. adusta*	2.5 mg/L	100% after 15 min	100% after 15 min	-	100% after 15 min	Eibes et al., 2011
	LAC from *M. thermophila*	5 mg/L	100% after 24 h with mediators	100% after 3 h without mediators	-	100% after 5 h without mediators	Lloret et al., 2010
Stirred batch reactor with the compound spiked in wastewater	LAC from *Trametes sp.*	100 ng/L	100% after 1 h	100% after 1 h	100% after 1 h	100% after 1 h	Auriol et al., 2007
	LAC from *T. versicolor*	100 ng/L	100% after 1 h	100% after 1 h	100% after 1 h	100% after 1 h	Auriol et al., 2008

Furthermore, the scenario regarding the conversion of E2 to E1 in soil remains both controversial and elusive. Taking into consideration the less effective removal of E1 in STPs (Johnson and Sumpter, 2001) and batch experiments (Suzuki and Maruyama, 2006) in comparison to E2, we can postulate that the bacteria capable of transforming E2 directly to non-estrogenic compounds not via E1 either are not so prevailing in activated sludge as the bacteria that are able to transform E2 to E1 but are not able to further oxidize E1, or are inhibited by other unknown factors in the mixed culture.

Neither autoclaving (Colucci et al., 2001) nor oven-drying at 85°C for 24h (Casey et al., 2003) was able to prevent the oxidation of E2 to E1 in soil. These results suggest that either the enzyme responsible for E2 transformation in soils is able to survive from autoclaving and oven-drying process, or oxidation of E2 to E1 can proceed abiotically. But they were contradictive to a recent finding of Mashtare et al. (2013), who reported that transformation of amended hormones in autoclaved sediments was markedly slower than in nonautoclaved sediments. This result was consistent with a previous study which showed that E2 was only oxidized to E1 via biological processes (Fan et al., 2007).

The discrepancy could be attributed to the difference of adopted experimental strategy. In contrast to the research of Fan et al. (2007), where 500 mg $HgCl_2$ per kg soil was added to the autoclaved soil, no inhibitor was used in the research of Colucci et al. (2001a) and Casey et al. (2003), where the inadvertent introduction of airborne microorganisms could not be ruled out when periodically sampling. Although it is not understood how the relative rates of biotic and abiotic degradation rates influence the transform of E2 to E1, E1 was shown to degrade primarily through microbial processes under aerobic conditions (Colucci et al., 2001a). More studies further confirmed that microbial processes play a key role in E2 mineralization under aerobic conditions supported by the fact that no mineralization of E2 was observed in sterile soils (Fan et al., 2007; Jacobsen et al., 2005).

With regard to E1 mineralization, two different pathways have been thus far proposed. In the mineralization study using ^{14}C-E2, which was labeled on the C-4 carbon, Layton et al. (2000) demonstrated that the conversion of E1 proceeded by a cleavage of the A-ring based on the release of ^{14}C-CO_2. The possible metabolite resulted from A-ring cleavage was suggested by an earlier study (Coombe et al., 1966) as showed in Fig. 1. Alternatively, Lee and Liu (2002) indicated that E2 was oxidized from the cyclopentane ring D at C17 into E1 during enzymatic degradation and then further degraded into metabolite X1 and finally to carbon dioxide through a tricarboxylic acid (TCA) cycle due to the detection of lactone as an intermediate. The pathway is given as Fig. 2. Additionally, dehydration was firstly reported as a mechanism of microbial estrogen degradation when Nakai et al. (2011) studied the degradation pathways of 17β-estradiol by *Nitrosomonas europaea* and determined whether the degradation products of 17β-estradiol had estrogenic activity. 1,3,5(10),16-Estratetraen-3-ol (estratetraenol) was newly recognized as a degradation intermediate produced by dehydration of 17β-estradiol. Its degradation rate was faster than that of 17β-estradiol. The yeast two-hybrid assay proved that estratetraenol acted as a ligand for the estrogen receptor; estratetraenol thus has potential estrogenic activity.

5.3. Degradation pathways of EE2

Degradation pathways of the natural estrogens as well as of EE2 are still controversial. In batch experiments with nitrifying activated sludge, Vader et al. (2000) found that EE2 was degraded completely within 6 days and an unidentified hydrophilic metabolite was produced and postulated that nitrifying bacteria were responsible for the steroid transformation by co-metabolism. Layton et al. (2000) indicated that the cleavage of the A-ring was involved in the mineralization of EE2 by conducting an experiment with ^{14}C-EE2 labeled on the C-4 carbon. Recently, three catabolic intermediates, including E1, resulted from EE2 degradation, were identified during a study carried out with *Sphingobacterium* sp. JCR5 isolated from activated sludge obtained from a wastewater treatment plant of an oral contraceptives producing factory. According to Haiyan et al. (2007), in the first step EE2 is oxygenized to three main catabolic intermediates, namely, E1, 2-hydroxy-2,4-dienevaleric acid and 2-hydroxy-2,4-diene-1,6-dioic acid. The second compound shares the analogous route with a previously reported testosterone-degrading bacterium and the latter is a metabolite with a different cleavage position from the former. It is conceivable that the degradation pathways vary from microbe to microbe.

Fig. 1. Suggested intermediates as a result of ring cleavage of estrone (E1)

Fig. 2. A proposed route of ring cleavage of estone (E1)

However, that the degradation of EE2 is firstly oxidized to E1 by the splitting of the ethinyl group suggested by Haiyan et al. (2007) is not in agreement with the study where two differently radiolabeled EE2 was applied continuously to a bench-scale membrane bioreactor with adapted industrial activated sludge (Cirja et al., 2007). A unique degradation product was identically detected in two experiments performed with the two differently radiolabeled EE2 labeled on the C-4 and C-20 carbon, respectively.

The authors consequently believed that the ethinyl group was not substituted due to no detection of ^{14}C-labelled ethinyl. Nevertheless, in terms of the downstream part of the whole degradation pathway, the oxidization of ethinyl group could not be ruled out due to that $^{14}CO_2$ was produced in both experiments using the different ^{14}C-EE2, though the percents of mineralization were both less than 1% (Cirja et al., 2007). Yi et al. (2007) suggested that ring A (aromatic ring) of EE2 was the site of electrophilic initiating reactions, including conjugation, hydroxylation and cleavage; due to electron density associated with ring A was significantly higher than that in other rings of EE2 , which could be useful for further efforts to elucidate biodegradation pathway of EE2. In contrast, Haiyan et al. (2007) proposed that EE2 was initially oxidized to E1, and that the pathway continued with ring opening oxidation reactions on ring B, leaving ring A initially intact based on the detected daughter products in an isolation culture experiment. It can be seen that the degradation pathway by co-metabolism is different with that by isolated bacterium being able to utilize EE2 as growth substrate. Future work must identify daughter products to investigate the metabolic details.

6. Cometabolic steroid degradation especially by nitrifying microorganisms

Cometabolic transformations are reactions that are catalyzed by existing microbial enzymes and that yield no carbon or energy benefits to the transforming cells. Therefore, a growth substrate must be available at least periodically to grow new cells, provide an energy source, and induce production of the cometabolic enzymes. Cometabolism- in which an organic compound is modified but not utilised for growth - is another important biodegradation process (Alexander, 1994), especially for micropollutants including estrogens present in significantly low concentration.

It is well documented that biodegradation of estrogens is clearly stimulated in the presence of nitrifying bacteria. Moreover, the enhancement was most probably due to co-metabolic effects. Several bacterial strains that produce monooxygenase enzymes are known to aerobically cometabolise organic compounds. *Nitrosomonas europaea* is a ubiquitous monooxygenase-producing bacterium catalyzing the oxidation of ammonium in soils, natural waters and nitrifying activated sludge (Shi et al., 2004b). It has often been demonstrated that ammonium monooxygenase (AMO) in the cells of *Nitrosomonas europaea* is capable of co-oxidising many organic compounds. Vader et al. (2000) used activated sludge in a laboratory reactor with ammonium and hydrazine as the energy sources and found approximately 28h half-life of EE2 by sludges that nitrified. The sludges that failed to nitrify significantly also failed to degrade EE2.

This result was afterwards supported by Shi et al. (2004a), who found that using allylthiourea (ATU), a chemical that inhibits the nitrification by blocking the ammonium monooxygenase (AMO), resulted in a decline of degradation rate for EE2 by about 80%, while that for E1 and E2 remained the same. Compared to the completely blocked in a culture of pure-strain *Nitrosomonas europaea* inhibited by ATU, the degradation of EE2 only slowed down in a mixed culture of nitrifiers and heterotrophs inhibited by ATU, which suggested that there were also other bacteria being able to convert EE2 in activated sludge (Shi et al., 2004a). It was demonstrated that the cometabolic degradation of E1, E2 and EE2 was the primary approach of their removal in nitrifying activated sludge and the activity of ammonia-

oxidizing bacteria dominating their degradation (Ren, et al., 2007). Although AMO may be inhibited by acetylene (Bollmann and Conrad, 1997; Teissier and Torre, 2002), the ethynyl group contained in EE2 did not appear to inhibit AMO activity (Yi et al., 2007). This conclusion was afterwards supported by Khunjar et al. (2011), who evaluated the biological fate of EE2 with flow through reactors containing an ammonia oxidizing bacterial (AOB) culture, two enriched heterotrophic cultures lack of nitrifier activity, and nitrifying activated sludge (NAS) cultures. It was found that EE2 removal slowed remarkably after AOBs were inhibited. It was worth noting that AOBs biotransformed EE2 five times faster than heterotrophs. Moreover, The cometabolic activity of AMO responsible for EE2 degradation is linearly correlated with ammonia oxidizing rate that usually regulated by initial ammonia concentration, temperature (Vader et al., 2000; Yi et al., 2006; Yi et al., 2007). Interestingly, Forrez et al. (2009) found that the elimination of EE2 was not affected by the absence of ammonium in the feed, suggesting that ammonia oxidizing bacteria (AOB) were able to maintain their population density and their activity, even after several months of starvation. Not only was AMO, but also other nonspecific monooxygenase enzymes were probably contributing to the degradation of estrogens. For instance, although the all 14 strains isolated by Yu et al. (2007) were capable of converting E2 to E1, only three of them (two strains of *Aminobacter* and one strain of *Sphingomonas*, all three strains belong to *Alphaproteobacteria*) showed the ability to degrade E1, which unexceptionally exhibited nonspecific monooxygenase activity rather than nonspecific dioxygenase activity.

Nonspecific monooxygenases are known to be responsible for metabolic and/or cometabolic reactions of a wide range of organics (Leahy et al., 2003; McCarty, 1997), including EE2 (Shi et al., 2004a). It is likely that nonspecific monooxygenase enzymes were responsible for estrogen degradation. It should be noted that the one strain of *Sphingomonas* mentioned above is unique with the ability to utilize E2 as a sole carbon source for growth. In other words, only one consortium of the fourteen isolates degraded E2 by growth linked metabolic reaction, while the other 13 isolates might via co-metabolic reaction. Afterwards, Roh (2009) found that *Sphingomonas* strain KC8 exhibited nonspecific monooxygenase activity but not dioxygenase activity via the results of enzymatic characterization. Nonspecific monooxygenase enzymes may be responsible for estrogen degradation by strain KC8, which supports the conclusion before.

Tran et al. (2013) suggested that cometabolism of EOCs (emerging trace organic contaminants), which includes estrogens, is widely noted in autotrophic microbes, such as autotrophic ammonia oxidizers via the non-specific enzymes, e.g. AMO. It is due to the fact that the autotrophic oxidizers utilize inorganic carbon and ammonia as the sole carbon and energy sources for their growth and induce AMO and cofactors as well. However, heterotrophic microbes can get involved in both cometabolism and/or metabolism depending on both the concentration of EOCs in the environment and their toxicity to the microbes.

Interestingly, a particular co-metabolic transformation process was recently reported occurring between natural and synthetic estrogens (Pauwels et al., 2008). With the intention of isolating EE2-degrading microorganisms from compost, Pauwels and co-workers (Pauwels et al., 2008) not only successfully isolated six strains, but also discovered that all six isolates were only able to co-metabolize EE2 in the presence of E2 or E1, possibly even E3, while fail to metabolize EE2 as the sole carbon sources. In the co-metabolism experiment using E2 and EE2 as coexistent substrate, It was observed that EE2 remained constant until the depletion of E2, and was subsequently degraded as soon as the E1 began to decrease, which early accumulated as a temporary intermediate. The authors speculated that certain enzyme, produced by the isolate, involved in degradation of E1 was perhaps responsible for the degradation of EE2 simultaneously. Apparently, more focused research is highly desired, due to the ubiquitous coexistence of E2, E1 and EE2 in urine, activated sludge, soil and compost, etc.

7. Conclusion and outlook

Generally, the sequence of degradation efficiency is E2 ＞ E1>>EE2. Although E2 is fast degraded to E1, this does not reduce the estrogenic potential remarkably. E1 is quite persistent in the environment, while EE2 is even more recalcitrant because it contains an ethinyl group at the same C-atom. According to research by far, most important degradation process is the degradation by microorganisms. *Cornybacterium* spp., *Nitrosomonas europaea* etc. are proved to be able to degrade estrogens. Moreover, the degradation rates can be influenced by temperature, pH values, redox conditions, etc. In general, better results can be gained at relatively higher temperatures. Bacteria that can degrade steroid hormones metabolically under denitrifying conditions could be isolated. It is however not certain if these bacteria have a big influence in removing estrogens, or if other bacteria in the denitrifying tank is in favor of the degradation.

Although research has been done to determine removal rates of estrogens, their degradation pathways are still dubious. The oxidation from E2 to E1, also called the first step, is widely accepted. Nevertheless, it is still unclear at which ring the cleavage initially occurs and which enzymes are involved in the estrogens degradation pathway. Further studies are required to determine this pathway. Moreover, pathways of EE2 are still controversial, as several incompatible theories have been proposed.

Lastly, before being put into practical application, the ability of the organisms to degrade estrogens needs to be enhanced. As the ambient

concentrations of estrogens in wastewater are much lower than the concentrations used in published studies, further studies are needed to address the prevalence of the isolates in biological treatment processes as well as to determine the significance of the isolates in estrogen removal. Quite a few aspects, including the ability to utilize other available organics and other estrogenic compounds in wastewater, the pathways, enzymes, and kinetics of estrogen degradation, have to be investigated in order to fully capitalize the degradation ability of these strains for potentiating estrogen degradation.

Acknowledgements
The present work was supported by the National Science Foundation of China (Grant No. 41571476 and No. 51109038), the Qing Lan Project of Jiangsu Province, and Startup Fund for Talented Scholars of Nanjing Normal University.

References

Adlercreutz H., Fotsis T., Bannwart C., Hamalainen E., Bloigu S., Ollus A., (1986), Urinary estrogen profile determination in young finnish vegetarian and omnivorous women, *Journal of Steroid Biochemistry*, **24**, 289-296.

Adlercreutz H., Gorbach S.L., Goldin B.R., Woods M.N., Dwyer J.T., Hamalainen E., (1994), Estrogen metabolism and excretion in Oriental and Caucasian women, *Journal of the National Cancer Institute*, **86**, 1076-1082.

Ainsworth L., Common R.H., Carter A.L., (1962), A chromatographic study of some conversion products of estrone-16-C-14 in the urine and feces of the laying hen, *Canadian Journal of Biochemistry and Physiology*, **40**, 123-135.

Andersen H., Siegrist H., Halling-Sørensen B., Ternes T.A., (2003), Fate of estrogens in a municipal sewage treatment plant, *Environmental Science & Technology*, **37**, 4021-4026.

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

Alexander M., (1994), *Biodegradation and Bioremediation*, Academic Press, San Diego, USA.

Auriol M., Filali-Meknassi Y., Tyagi R.D., Adams C.D., (2007), 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., Piña 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.

Balaguer P., Francois F., Comunale F., Fenet H., Boussioux A.M., Pons M., Nicolas J.C., Casellas C., (1999), Reporter cell lines to study the estrogenic effects of xenoestrogens, *Science of the Total Environment*, **233**, 47-56.

Balkwill D.L., Drake G.R., Reeves R.H., Fredrickson J.K., White D.C., Ringelberg D.B., Chandler D.P., Romine M.F., Kennedy D.W., Spadoni C.M., (1997), Taxonomic study of aromatic-degrading bacteria from deep-terrestrial-subsurface sediments and description of *Sphingomonas aromaticivorans* sp. nov., *Sphingomonas subterranea* sp. nov., and *Sphingomonas stygia* sp. nov. *International Journal of Systematic and Evolutionary Microbiology*, **47**, 191-201.

Baronti C., Curini R., D'Ascenzo G., Di Corcia A., Gentili A., Samperi R., (2000), Monitoring natural and synthetic estrogens at activated sludge sewage treatment plants and in a receiving river water, *Environmental Science & Technology*, **34**, 5059-5066.

Belhaj D., Jaabiri I., Ayadi H., Kallel M., Zhou J.L., (2013), Occurrence and removal of steroidal estrogens in Centre Eastern Tunisia municipal sewage treatment plant, *Desalination and Water Treatment*, **14**, 2330-2339.

Berthe-Corti L., Nachtkamp M., (2010), *54 Bacterial Communities in Hydrocarbon-Contaminated Marine Coastal Environments*, In: *Handbook of Hydrocarbon and Lipid Microbiology*, Kenneth N., Timmis (Eds.), Springer, Berlin Heidelberg New York, 2349-2359.

Bhushan B., Samanta S.K., Chauhan A., Chakraborti A.K., Jain R.K., (2000), Chemotaxis and biodegradation of 3-methyl-4-nitrophenol by *Ralstonia* sp. SJ98, *Biochemical and Biophysical Research Communications*, **275**, 129-133.

Bollmann A., Conrad R., (1997), Recovery of nitrification and production of NO and N_2O after exposure of soil to acetylene, *Biology and Fertility of Soils*, **25**, 41-46.

Bolton J.L., Pisha E., Zhang F., Qiu S., (1998), Role of quinoids in estrogen carcinogenesis, *Chemical Research in Toxicology*, **11**, 1113-1127.

Cajthaml T., (2015), Biodegradation of endocrine-disrupting compounds by ligninolytic fungi: mechanisms involved in the degradation, *Environmental Microbiology*, **17**, 4822-4834.

Casey F.X.M., Larsen G.L., Hakk H., Simunek J., (2003), Fate and transport of 17*β*-estradiol in soil-water system, *Environmental Science & Technology*, **37**, 2400-2409.

Castellana G., Loffredo E., (2014), Simultaneous removal of endocrine disruptors from a wastewater using white rot fungi and various adsorbents, *Water, Air & Soil Pollution*, **225**, 1-13.

Chen M.X., Fang H., Qi R., He S.X., Wei Y.S., (2016), 17β-estradiol degrading bacteria in sequencing batch reactors for swine wastewater treatment, *Environmental Engineering and Management Journal*, **15**, 2273-2278.

Chimchirian R.F., Suri R.P.S., Fu H.X., (2007), Free synthetic and natural estrogen hormones in influent and effluent of three municipal wastewater treatment plants, *Water Environment Research*, **79**, 969-974.

Chun S., Lee J., Geyer R., White D.C., Raj Raman D., (2005), Effect of agricultural antibiotics on the persistence and transformation of 17β-Estradiol in a sequatchie loam, *Journal of Environmental Science and Health, Part B: Pesticides, Food Contaminants, and Agricultural Wastes*, **40**, 741-751.

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

Colucci M.S., Bork H., 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.

Coombe R.G., Tsong Y.Y., Hamilton P. B., Sih C.J., (1966), Mechanisms of steroid oxidation by microorganisms. X. Oxidative cleavage of estrone, *Journal of Biological Chemistry*, **241**, 1587-1595.

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

Das B.S., Lee L.S., Rao P.S.C., Hultgren R.P., (2004), Sorption and degradation of steroid hormones in soils during transport: Column studies and model evaluation, *Environmental Science & Technology*, **38**, 1460-1470.

de Mes, T., Zeeman G., Lettinga G., (2005), Occurrence and fate of estrone, 17β-estradiol and 17α-ethynylestradiol in STPs for domestic wastewater, *Reviews in Environmental Science and Bio/Technology*, **4**, 275-311.

Desbrow C., Routledge E.J., Brighty G.C., Sumpter J.P., Waldock M., (1998), Identification of estrogenic chemicals in STW effluent. 1. Chemical fractionation and *in vitro* biological screening, *Environmental Science & Technology*, **32**, 1549-1558.

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.

Eibes G., Debernardi G., Feijoo G., Moreira M. T., Lema J.M., (2011), Oxidation of pharmaceutically active compounds by a ligninolytic fungal peroxidase, *Biodegradation*, **22**, 539-550.

Esperanza M., Suidan M.T., Nishimura F., Wang Z.M., Sorial G.A., (2004), Determination of sex hormones and nonylphenol ethoxylates in the aqueous matrixes of two pilot-scale municipal wastewater treatment plants, *Environmental Science & Technology*, **38**, 3028-3035.

Essandoh H.M.K., Tizaoui C., Mohamed M.H.A., (2012), Removal of estrone (E1), 17β-estradiol (E2) and 17α-ethinylestradiol (EE2) during soil aquifer treatment of a model wastewater, *Separation Science and Technology*, **47**, 777-787.

Fahrbach M., Kuever J., Meinke R., Kämpfer P., Hollender J., (2006), Denitratisoma oestradiolicum gen. nov., sp. nov., a 17β-oestradiol-degrading, denitrifying betaproteobacterium, *International Journal of Systematic and Evolutionary Microbiology*, **56**, 1547-1552.

Fan Z., Casey F.X.M., Hakk H., Larsen G.L., (2007), Persistence and fate of 17β-estradiol and testosterone in agricultural soils, *Chemosphere*, **67**, 886-895.

Fotsis T., Järvenpää P., Adlecreutz H., (1980), Purification of urine for quantification of the complete estrogen profile, *Journal of Steroid Biochemistry*, **12**, 503-508.

Frederickson J.K., Balkwill D.L., Drake G.R., Romine M.F., Ringelberg D.B., White D.C., (1995), Aromatic-degrading *Sphingomonas* isolates from the deep substrate, *Applied and Environmental Microbiology*, **61**, 1917-1922.

Fujii K., Kikuchi S., Satomi M., Ushio-Sata N., Morita N., (2002), Degradation of 17*β*-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.

Fujii K., Satomi M., Morita N., Motomura T., Tanaka T., Kikuchi S., (2003), *Novosphingobium tardaugens* sp. nov., an oestradiol-degrading bacterium isolated from activated sludge of a sewage treatment plant in Tokyo, *International Journal of Systematic and Evolutionary Microbiology*, **53**, 47-52.

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

Gesell M., Hammer E., Specht M., Francke W., Schauer F., (2001), Biotransformation of biphenyl by *Paecilomyces lilacinu*s and characterization of ring cleavage products, *Applied and Environmental Microbiology*, **67**, 1551-1557.

Hakk H., Millner P., Larsen G., (2005), Decrease in water-soluble 17β-estradiol and testosterone in composted poultry manure with time, *Journal of Environmental Quality*, **34**, 943-950.

Haiyan R., Shulan J., ud din Ahmad N., (2007), Degradation characteristics and metabolic pathway of 17α-ethynylestradiol by *Sphingobacterium* sp. JCR5, *Chemosphere*, **66**, 340-346.

Hashimoto T., Onda K., Nakamura Y., Tada K., Miya A., Murakami T., (2007), Comparison of natural estrogen removal efficiency in the conventional activated sludge process and the oxidation ditch process, *Water Research*, **41**, 2117-2126.

Hashimoto T., Onda K., Morita T., Luxmy B.S., Tada K., Miya A., Murakami T., (2009), Contribution of the estrogen-degrading bacterium *Novosphingobium* sp. strain JEM-1 to estrogen removal in wastewater treatment, *Journal of Environmental Engineering*, **136**, 890-896.

Herman J.S., Mills A.L., (2003), Biological and hydrological interactions affect the persistence of 17β-estradiol in an agricultural watershed, *Geobiology*, **1**, 141-151.

Hilakivi-Clarke L., de Assis S., Warri A., (2013), Exposures to synthetic estrogens at different times during the life, and their effect on breast cancer risk, *Journal of Mammary Gland Biology and Neoplasia*, **18**, 25-42.

Hoffmann B., Depinho T.G., Schuler G., (1997), Determination of free and conjugated oestrogens in peripheral blood plasma, feces and urine of cattle throughout pregnancy, *Experimental and Clinical Endocrinology Diabetes*, **105**, 296-303.

Hutchins S.R., White M.V., Hudson F.M., Fine D.D., (2007), Analysis of lagoon samples from different concentrated animal feeding operations for estrogens and estrogen conjugates, *Environmental Science & Technology*, **41**, 738-744.

Ivie G.W., Christopher R.J., Munger C.E., Coppock C.E., (1986), Fate and residues of [4-^{14}C] estradiol-17 beta after intramuscular injection into Holstein steer calves, *Journal of Animal Science*, **62**, 681-690.

Jacobsen A., Lorenzen A., Chapman R., Topp E., (2005), Persistence of testosterone and 17β-estradiol in soils receiving swine manure or municipal biosolids, *Journal of Environmental Quality*, **34**, 861-871.

Jiang L., Yang J., Chen J., (2010), Isolation and characteristics of 17β-estradiol-degrading *Bacillus* spp. strains from activated sludge, *Biodegradation*, **21**, 729-736.

Johnson A.C., Belfroid A., Di Corcia A., (2000), Estimating steroid oestrogen inputs into activated sludge treatment works and observations on their removal from the effluent, *Science of the Total Environment*, **256**, 163-173.

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.

Jürgens 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.

Ke J., Zhuang W., Gin K.Y., 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.

Khunjar W.O., Mackintosh S.A., Skotnicka-Pitak J., Baik S., Aga D.S., Love N.G., (2011), Elucidating the relative roles of ammonia oxidizing and heterotrophic bacteria during the biotransformation of 17α-ethinylestradiol and trimethoprim, *Environmental Science & Technology*, **45**, 3605-3612.

Knight W.M., (1980), Estrogens administered to food producing animals: environmental considerations, *Estrogens in the Environment*, Elsevier/North-Holland, New York, 391-402.

Körner W., Spengler P., Bolz U., Schuller W., Hanf V., Metzger J.W., (2001), Substances with estrogenic activity in effluents of sewage treatment plants in southwestern Germany. 2. Biological analysis, *Environmental Toxicology and Chemistry*, **20**, 2142-2151.

Kuch H.M., Ballschmitter K., (2001), Determination of endocrine-disrupting phenolic compounds and estrogens in surface and drinking water by HRGC-(NCI)-MS in the picogram per liter range, *Environmental Science & Technology*, **35**, 3201-3206.

Kumar A.K., Sarma P.N., Venkata Mohan S.R., (2016), Incidence of selected endocrine disrupting estrogens in water bodies of Hyderabad and its relation to water quality parameters, *Environmental Engineering and Management Journal*, **15**, 315-325.

Kurisu F., Ogura M., Saitoh S., Yamazoe A., Yagi O., (2010), Degradation of natural estrogen and identification of the metabolites produced by soil isolates of *Rhodococcus* sp. and *Sphingomonas* sp., *Journal of Bioscience and Bioengineering*, **109**, 576-582.

Kuster M., López de Alda M.J., Barceló D., (2004), Analysis and distribution of estrogens and progestogens in sewage sludge, soils and sediments, *Trends in Analytical Chemistry*, **23**, 790-798.

Lai K.M., Johnson K.L., Scrimshaw M.D., Lester J.N., (2000), Binding of waterborne steroid estrogens to solid phases in river and estuarine systems, *Environmental Science & Technology*, **34**, 3890-3894.

Lange R., Hutchinson T.H., Croudace C.P., Siegmund F., Schweinfurth H., Hampe P., Panter G.H., Sumpter J.P., (2001), Effects of the synthetic estrogen 17α-ethinylestradiol on the life-cycle of the fathead minnow (*Pimephales promelas*), *Environmental Toxicology and Chemistry*, **20**, 1216-1227.

Larcher S., Yargeau V., (2013), Biodegradation of 17α-ethinylestradiol by heterotrophic bacteria, *Environmental Pollution*, **173**, 17-22.

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 U.S.A, *Environmental Science & Technology*, **34**, 3925-3931.

Leahy J.G., Batchelor P.J., Morcomb S.M., (2003), Evolution of the soluble diiron monooxygenases, *FEMS Microbiology Reviews*, **27**, 449-479.

Lee H.B., Liu D., (2002), Degradation of 17*β*-estradiol and its metabolites by sewage bacteria, *Water Air and Soil Pollution*, **134**, 353-368.

Legler J., (2001), *Development and application of in vitro and in vivo reporter gene assays for the assessment of (xeno-) estrogenic compounds in the aquatic environment*, PhD Thesis, Wageningen Universiteit, On line at: https://edepot.wur.nl/199007.

Li G.P., Yang Z.Y., He Z., Chen M., Yang X.L., (2013a), Optimization of solid-phase extraction for determining estrone in sewage by response surface methodology, *Environmental Science & Technology*, **3**, 026.

Lloret L., Eibes G., Lú-Chau T.A., Moreira M.T., Feijoo G., Lema J.M., (2010), Laccase-catalyzed degradation of anti-inflammatories and estrogens, *Biochemical Engineering Journal*, **51**, 124-131.

Lucas S.D., Jones D.L., (2006), Biodegradation of estrone and 17β-estradiol in grassland soils amended with animal wastes, *Soil Biology and Biochemistry*, **38**, 2803-2815.

Maeda M., Chung S.Y., Song E., Kudo T., (1995), Multiple genes encoding 2,3-dihydroxybiphenyl 1,2-dioxygenase in the gram-positive polychlorinated biphenyl-degrading bacterium *Rhodococcus erythropolis* TA421, isolated from a termite ecosystem, *Applied and Environmental Microbiology*, **61**, 549-555.

Maeng S.K., Choi B.G., Lee K.T., Song K.G., (2013), Influences of solid retention time, nitrification and microbial activity on the attenuation of pharmaceuticals and estrogens in membrane bioreactors, *Water research*, **47**, 3151-3162.

Mashtare M.L., Lee L.S., Nies L.F., Turco R.F., (2013), Transformation of 17α-Estradiol, 17β-Estradiol, and estrone in sediments under nitrate-and sulfate-reducing conditions, *Environmental Science & Technology*, **47**, 7178-7185.

Matsui S., Takigami H., Matsuda T., Taniguchi N., Adachi J., Kawami H., Shimizu Y., (2000), Estrogen and estrogen mimics contamination in water and the role of sewage treatment, *Water Science & Technology*, **42**, 173-179.

McCarty P.L., (1997), Breathing with chlorinated solvents, *Science*, **276**, 1521-1522.

Moschet C., (2009), *Microbial degradation of steroid hormones in the environment and technical systems*, PhD Thesis, Institute of Technology, Institute of Biogeochemistry and Pollutant Dynamics (IBP-ETH), Swiss.

Nakai S., Yamamura A., Tanaka S., Shi J., Nishikawa M., Nakashimada Y., Hosomi M., (2011), Pathway of 17β-estradiol degradation by *Nitrosomonas europaea* and reduction in 17β-estradiol-derived estrogenic activity, *Environmental Chemistry Letters*, **9**, 1-6.

Nakamura A., Tamura I., Takanobu H., Yamamuro M., Iguchi T., Tatarazako N., (2014), Fish multigeneration test with preliminary short- term reproduction assay for estrone using Japanese medaka (*Oryzias latipes*), *Journal of Applied Toxicology*, **35**, 11-30.

Nohynek L.J., Nurmiaho-Lassila E.L., Suhonen E.L., Busse H.J., Mohammadi M., Hantula J., Rainey F., Salkinoja-Salonen M.S., (1996), Description of chlorophenol-degrading *Pseudomonas* sp. strains KF1T, KF3,and NKF1 as a new species of the genus *Sphingomonas,*

Sphingomonassubarctica sp. nov, *International Journal of Systematic Bacteriology*, **46**, 1042-1055.

Onda K., Nakamura Y., Takatoh C., Miya A., Katsu Y., (2003), The behavior of estrogenic substances in the biological treatment process of sewage, *Water Science & Technology*, **47**, 109-116.

Owen L., Keevil B., (2013), A rapid and sensitive LC-MS/MS assay for the routine analysis of estradiol and estrone, *Endocrine Abstracts*, **31**, P35, DOI: 10.1530/endoabs.31.P35.

Pakert M., Filipov E., Kunst S., (2003), Discharge of estrogens from sewage treatment plants II: Behavior during the digestion process, estimation of estrogen exposure (in German); poster 7, POSEIDON Symposium, On line at: http://www.bafg.de/portale/poseidon/Abstract_Book_Braunschweig_final.pdf.

Palme R., Fischer P., Schildorfer H., Ismail M.N., (1996), Excretion of infused 14C-steroid hormones via faeces and urine in domestic livestock, *Animal Reproduction Science*, **43**, 43-63.

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

Peterson E.W., Davis R.K., Orndorff H.A., (2000), 17 β-estradiol as an indicator of animal waste contamination in Mantled Karst Aquifers, *Journal of Environmental Quality*, **29**, 826-834.

Pillon A., Servant N., Vignon F., Balaguer P., Nicolas J.C., (2005), *In vivo* bioluminescence imaging to evaluate estrogenic activities of endocrine disrupters, *Analytical Biochemistry*, **340**, 295-302.

Preda C., Ungureanu M.C., Vulpoi C., (2012), Endocrine disruptors in the environment and their impact on human health, *Environmental Engineering and Management Journal*, **11**, 1697-1706.

Raman D.R., Layton A.C., Moody L.B., Easter J.P., Sayler G.S., Burns R.T., Mullen M.D., (2001), Degradation of estrogens in dairy waste solids: effect of acidification and temperature, *Transactions of the ASAE*, **44**, 1881-1888.

Raman D.R., Williams E.L., Layton A.C., Burns R.T., Easter J.P., Daugherty A.S., Mullen M.D., Sayler G.S., (2004), Estrogen content of dairy and swine wastes, *Environmental Science & Technology*, **38**, 3567-3573.

Rodgers-Gray T.P., Jobling S., Morris S., Kelly C., Kirby S., Janbakhsh A., Harries J.E., Waldock M.J., Sumpter J.P., Tyler C.R., (2000), Long-term temporal changes in the estrogenic composition of treated sewage effluent and its biological effects on fish, *Environmental Science & Technology*, **34**, 1521-1528.

Roh H.K., (2009), *Deciphering active estrogen-degrading microorganisms in bioreactors*, PhD Thesis, Texas A&M University, Texas, USA.

Roh H., Chu K.H., (2010), A 17β-estradiol-utilizing bacterium, *Sphingomonas* strain KC8: Part I-Characterization and abundance in wastewater treatment plants, *Environmental Science & Technology*, **44**, 4943-4950.

Rose K.P., Farenhorst A., (2014), Estrone and 17β-estradiol mineralization in liquid swine manure and soil in the presence and absence of penicillin or tetracycline, *Journal of Environmental Science and Health, Part B*, **49**, 331-337.

Routledge E.J., Sheahan D., Desbrow C., Brighty G.C., Waldock M., Sumpter J.P., (1998), Identification of estrogenic chemicals in STW effluent: 2. In vivo responses in trout and roach, *Environmental Science & Technology*, **32**, 1559-1565.

Ruchiraset A., Chinwetkitvanich S., (2014), Estrogens removal by sludge from enhance biological phosphorus removal system, *Advanced Materials Research*, **931**, 246-250.

Sang Y., Xiong G., Maser E., (2012), Identification of a new steroid degrading bacterial strain H5 from the Baltic Sea and isolation of two estradiol inducible genes, *The Journal of Steroid Biochemistry and Molecular Biology*, **129**, 22-30.

Sarmah A.K., Northcott G.L., Leusch F.D.L., Tremblay L.A., (2006), A survey of endocrine disrupting chemicals (EDCs) in municipal sewage and animal waste effluents in the Waikato region of New Zealand, *Science of the Total Environment*, **355**, 135-144.

Sarah C., Guillermina H.R., (2010), Occurrence, fate, and biodegradation of estrogens in sewage and manure, *Applied Microbiology and Biotechnology*, **86**, 1671-1692.

Schlenker G., Birkelbach C., Glatzel P.S., (1999a), Analysis of the influence of temperature on the stability of sex steroids in cow feces, *Berliner und Muenchener Tieraerztliche Wochenschrift*, **112**, 459-464.

Schlenker G., Muller W., Birkelbach C., Glatzel P.S., (1999b), Experimental investigations into influence of *Escherichia coli* and *Clostridium perfringens* on the steroid estrone, *Berliner und Muenchener Tieraerztliche Wochenschrift*, **112**, 14-17.

Schlenker G., Muller W., Glatzel P., (1998), Analysis for the stability of sexual steroids in feces of cows over 12 weeks, *Berliner und Muenchener Tieraerztliche Wochenschrift*, **111**, 248-252.

Shi J., Chen Q., Liu X., Zhan X., Li J., Li Z., (2013), Sludge/water partition and biochemical transformation of estrone and 17β-estradiol in a pilot-scale step-feed anoxic/oxic wastewater treatment system, *Biochemical Engineering Journal*, **74**, 107-114.

Shi J., Fujisawa S., Nakai S., Hosomi M., (2004a), 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., Nakai S., Hosomi M., (2004b), Microbial degradation of estrogens using activated sludge and night soil-compositing microorganisms, *Water Science & Technology*, **50**, 153-159.

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

Shin S.K., Kim J.E., Kwon G.S., Kwon J.W., Oh E.T., So J.S., Koh S.C., (2003), Isolation and identification of a pentachloronitrobenzene(PCNB) degrading bacterium *Alcaligenes xylosoxidans* PCNB-2 from agricultural soil, *Journal of Microbiology*, **41**,165-168.

Shore L.S., Harel-Markowitz E., Gurevich M., Shemesh M., (1993), Factors affecting the concentration of testosterone in poultry litter, *Journal of Environmental Science and Health A*, **28**, 1737-1749.

Souissi Y., Kinani S., Bouchonnet S., Bourcier S., Malosse C., Sablier M., Aït-Aïssa S., (2014), Photolysis of estrone generates estrogenic photoproducts with higher activity than the parent compound, *Environmental Science and Pollution Research*, **21**, 7818-7827.

Stumpe B., Marschner B., (2007), Long-term sewage sludge application and wastewater irrigation on the mineralization and sorption of 17β-estradiol and

testosterone in soils, *Science of the Total Environment*, **374**, 282-291.

Sumpter J.P., (1998), Reproductive effects from oestrogen activity in polluted water, *Archives of Toxicology*, **20**, 143-150.

Suzuki Y., Maruyama T., (2006), Fate of natural estrogens in batch mixing experiments using municipal sewage and activated sludge, *Water Research*, **40**, 1061-1069.

Szarka S., Nguyen V., Prokai L., Prokai-Tatrai K., (2013), Separation of dansylated 17β-estradiol, 17α-estradiol, and estrone on a single HPLC column for simultaneous quantitation by LC–MS/MS, *Analytical and Bioanalytical Chemistry*, **405**, 3399-3406.

Takeuchi M., Hamana K., Hiraishi A., (2001), Proposal of the genus *Sphingomonas* sensu stricto and three new genera, *Sphingobium*, *Novosphingobium*, and *Sphingopyxis*, on the basis of phylogenetic and chemotaxonomic analyses, *International Journal of Systematic and Evolutionary Microbiology*, **51**, 1405-1417.

Tan D.T., Arnold W.A., Novak P.J., (2013), Impact of organic carbon on the biodegradation of estrone in mixed culture systems, *Environmental Science & Technology*, **47**, 12359-12365.

Teissier S., Torre M., (2002), Simultaneous assessment of nitrification and denitrification on freshwater epilithic biofilms by acetylene block method, *Water Research*, **36**, 3803-3811.

Ternes T.A., (1998), Occurrence of drugs in German sewage treatment plants and rivers, *Water Research*, **32**, 3245-3260.

Ternes T.A., Kreckel P., Mueller J., (1999a), 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.

Ternes T.A., Stumpf M., Mueller J., Haberer K., Wilken R.D., Servos M., (1999b), Behavior and occurrence of estrogens in municipal sewage treatment plants. I. Investigations in Germany, Canada and Brazil, *Science of the Total Environment*, **225**, 81-90.

Tran N.H., Urase T., Ngo H.H., Hu J., Ong S.L., (2013), Insight into metabolic and cometabolic activities of autotrophic and heterotrophic microorganisms in the biodegradation of emerging trace organic contaminants, *Bioresource Technology*, **146**, 721-731.

Turfitt G.E., (1947), Microbiological agencies in the degradation of steroids II. Steroid utilization by the microflora of soils, *Journal of Bacteriology*, **54**, 557-562.

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.

Villemur R., dos Santos S.C.C., Ouellette J., Juteau P., Lépine F., Déziel E., (2013), Biodegradation of endocrine disruptors in solid-liquid two-phase Partitioning Systems By Enrichment Cultures, *Applied and Environmental Microbiology*, **79**, 4701-4711.

Weber S., Leuschner P., Kämpfer 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.

Wijekoon K.C., Hai F.I., Kang J., Price W.E., Guo W., Ngo H.H., Nghiem L.D., (2014), A novel membrane distillation-thermophilic bioreactor system: Biological stability and trace organic compound removal, *Bioresource Technology*, **159**, 334-341.

Williams C.L., Stancel G.M., (1996), *Estrogens and Progestins*, In: *The Pharmacological Basis of Therapeutics*, Hardman J.G., Limbird L.E., Molinoff P.B., Ruddon R.W., Goodman-Gilman A. (Eds), The McGraw-Hill Companies, USA, 1411-1440.

Writer J.H., Ryan J.N., Keefe S.H., Barber L.B., (2011), Fate of 4-nonylphenol and 17β-estradiol in the Redwood River of Minnesota, *Environmental Science & Technology*, **46**, 860-868.

Xu Y., Xu N., Llewellyn N. R., Tao H., (2014), Occurrence and removal of free and conjugated estrogens in wastewater and sludge in five sewage treatment plants, *Environmental Science: Processes & Impacts*, **16**, 262-270.

Xuan R.C., Blassengale A.A., Wang Q.Q., (2008), Degradation of estrogenic hormones in a silt loam soil, *Journal of Agricultural and Food Chemistry*, **56**, 9152-9158.

Yang S., Hai F.I., Nghiem L.D., Price W.E., Roddick F., Moreira M.T., Magram S.F., (2013), Understanding the factors controlling the removal of trace organic contaminants by white-rot fungi and their lignin modifying enzymes: a critical review, *Bioresource Technology*, **141**, 97-108.

Yazdi M.T., Malekzadeh F., Zarrini G., Faramarzi M.A., Kamranpour N., Khaleghparast S., (2001), Production of cholesterol oxidase by a newly isolated *Rhodococcus* sp., *World Journal of Microbiology and Biotechnology*, **17**, 731-737.

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

Yi T., Harper W.F., (2007), The link between nitrification and biotransformation of 17α-ethiny lestradiol, *Environmental Science & Technology*, **41**, 4311-4316.

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., (2005), Sorption and degradation of estrogen-like-endocrine disrupting chemicals in soil, *Environmental Toxicology and Chemistry*, **24**, 2640-2645.

Ying G.G., Kookana R.S., Ru Y.J., (2002), Occurrence and fate of hormone steroids in the environment, *Environment International*, **28**, 545-551.

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.

Yoon J.H., Cho Y.G., Kang S.S., Kim S.B., Lee S.T., Park Y.H., (2000), *Rhodococcus koreensis* sp. nov., a 2,4-dinitrophenol-degrading bacterium, *International Journal of Systematic and Evolutionary Microbiology*, **50**, 1193-1201.

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.

Yu C.P., Roh H., Chu K.H., (2007), 17*β*-estradiol-degrading bacteria isolated from activated sludge, *Environmental Science & Technology*, **41**, 486-492.

Yu Z., Huang W., (2005), Competitive sorption between 17α-ethinylestradiol and naphthalene/phenanthrene by

sediments, *Environmental Science & Technology*, **39**, 4878-4885.

Zeng Q., Li Y., Yang S., (2013), Sludge retention time as a suitable operational parameter to remove both estrogen and nutrients in an anaerobic–anoxic–aerobic activated sludge system, *Environmental Engineering Science*, **30**, 161-169.

Zhang T., Xiong G., Maser E., (2011), Characterization of the steroid degrading bacterium S19-1 from the Baltic Sea at Kiel, Germany, *Chemico-Biological Interactions*, **191**, 83-88.

Zhang X., Li X., Zhang Q., Peng Q., Zhang W., Gao F., (2014), New insight into the biological treatment by activated sludge: The role of adsorption process, *Bioresource Technology*, **153**, 160-164.

Zhang Z., Gao P., Su H., Zhan P., Ren N., Feng Y., (2013), Anaerobic biodegradation characteristics of estrone, estradiol, and 17 α-ethinylestradiol in activated sludge batch tests, *Desalination and Water Treatment*, **53**, 985-993.

Ziels R.M., Lust M.J., Gough H.L., Strand S.E., Stensel H.D., (2014), Influence of bioselector processes on 17α-ethinylestradiol biodegradation in activated sludge wastewater treatment systems, *Environmental Science & Technology*, **48**, 6160-6167.

Zou Y., Zhang Z., Shao X., Chen Y., Wu X., Yang L., Zhang D., (2013), Hollow-fiber-supported liquid-phase microextraction using an ionic liquid as the extractant for the preconcentration of bisphenol A, 17-β-estradiol, estrone and diethylstilbestrol from water samples with HPLC detection, *Water Science & Technology*, **69**, 1028-1035.

Zuo Y., Zhang K., Zhou S., (2013), Determination of estrogenic steroids and microbial and photochemical degradation of 17α-ethinylestradiol (EE2) in lake surface water, a case study, *Environmental Science: Processes & Impacts*, **15**, 1529-1535.