



# Biotransformation strategies for steroid estrogen and androgen pollution

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## Abstract

The common steroid hormones are estrone (E1), 17 $\beta$ -estradiol (E2), estriol (E3), 17 $\alpha$ -ethinylestradiol (EE2), and testosterone (T). These steroids are reported to contaminate the environment through wastewater treatment plants. Steroid estrogens are widespread in the aquatic environment and therefore pose a potential risk, as exposure to these compounds has adverse impacts on vertebrates. Excessive exposure to steroid estrogens causes endocrine disruption in aquatic vertebrates, which affects the normal sexual life of these animals. Steroid pollutants also cause several health problems in humans and other animals. Microbial degradation is an efficient method for removing hormone pollutants from the environment by remediation. Over the last two decades, microbial metabolism of steroids has gained considerable attention due to its higher efficiency to reduce pollutants from the environment. The present review is focused on the major causes of steroid pollution, concentrations of these pollutants in surface water, groundwater, drinking water, and wastewater, their effect on humans and aquatic animals, as well as recent efforts by various research groups that seek better ways to degrade steroids by aerobic and anaerobic microbial systems. Detailed overview of aerobic and anaerobic microbial biotransformation of steroid estrogens and testosterone present in the environment along with the active enzyme systems involved in these biotransformation reactions is described in the review article, which helps readers to understand the biotransformation mechanism of steroids in depth. Other measures such as co-metabolic degradation, consortia degradation, algal, and fungal steroid biotransformation are also discussed in detail.

**Keywords** Estrogen · Androgen · Aerobic and anaerobic biotransformation · Endocrine disrupter · Microbial metabolism

## Introduction

Steroid hormones directly interact with the normal functioning of endocrine systems, thus affecting reproduction and development in aquatic wildlife (Kumar et al. 2012). Some common steroid hormones (natural and synthetic) found in the environment include (E1), 17 $\beta$ -estradiol (E2), estriol (E3), 17 $\alpha$ -ethinylestradiol (EE2), mestranol (MeEE 2), and testosterone (T) (Table 1) (Ting and Praveena 2017). Both natural

and synthetic estrogens have a tetracyclic network consisting of a phenolic, two cyclohexane, and one cyclopentane ring. The difference in the configurations of the D ring at the C16 and 17 positions of estrogens, give rise to different compounds (Hamid and Eskicioglu 2012). Testosterone also has cyclopentanoperhydrophenanthrene nucleus as in estrogenic substances. Steroid hormones enter the environment through human and animal excretions (urine and feces) as well as from waste generated by pharmaceutical industries (Zheng et al. 2008). Waste containing estrogens are carried with wastewater into wastewater treatment plants (WWTPs) and then released into the aquatic environment, including drinking water (Sang et al. 2012), as WWTPs without biological treatment facilities are unable to efficiently remove estrogens (Ting and Praveena 2017). Increasing industrialization and modernization means that more estrogen would end up contaminating the aquatic environments (Jiang et al. 2013), thereby interfering with sexual development and reproduction in aquatic animals (Yu et al. 2013). Many studies have reported that the feminization of

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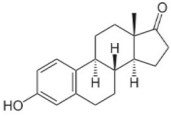
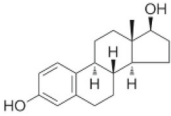
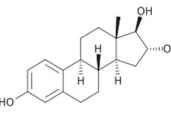
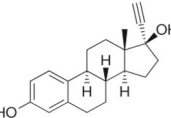
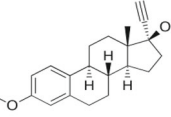
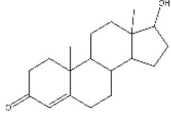
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**Table 1** Description of major natural and synthetic hormone found in environment

Name of EDCs	Molecular Weight	Molecular Formula	Molecular Structure
Estrone, E1	270.4	$C_{18}H_{22}O_2$	
17β-Estradiol, E2	272.4	$C_{18}H_{24}O_2$	
Estriol, E3	288.4	$C_{18}H_{24}O_3$	
17α-Ethinylestradiol, EE2	296.4	$C_{20}H_{24}O_2$	
Mestranol , MeEE 2	310.43	$C_{21}H_{26}O_2$	
Testosterone, T	288.4	$C_{19}H_{28}O_2$	

male fish is a result of estrogen contamination (Liu et al. 2016). Estrogen pollution affects intracellular estrogen receptors (ERs), which regulate the transcription of responsive genes, resulting in the initiation of rapid, non-genomic reactions. Malfunctional ERs disrupt the normal functioning of the estrogen system and causes defective homeostasis in fishes (Pinto et al. 2014). In aquatic systems, estrogens also act as endocrine-disrupting compounds (EDCs) because they interfere with the normal functioning of the endocrine system of aquatic animals by mimicking, and antagonizing the effect of endogenous hormones as well as disrupting the anabolism and catabolism of endogenous hormones (Silva et al. 2012; Liu et al. 2016). Estrogen contaminants also affect the immune, cardiovascular, and neurological systems in humans (McKinlay et al. 2008; Chighizola and Meroni 2012; Woclawek-Potocka et al. 2013). For instance, in humans,

exposure to endogenous or exogenous estrogenic compounds leads to lower sperm count, declining male reproductive health, and feminization of men (Sumpter and Jobling 2013). Excessive exposure to estrogen contamination also leads to susceptibility to several types of cancer such as prostate cancer in men and breast cancer in women (Trevino et al. 2015; Adeel et al. 2017). Some studies have also revealed that intake of a single estrogen or multiple estrogens in combination with progesterone lowers the intraocular pressure (IOP) inside the eye, which could damage the optical nerve and therefore lead to glaucoma (Adeel et al. 2017). Apart from estrogens, environmental androgen, i.e., testosterone, is a potential micropollutant, which interferes with the endocrine systems of organisms, even in trace amounts (Davis et al. 2000; Seki et al. 2004; Fu et al. 2019). Moreover, androgen can interfere with the reproductive development of aquatic

animals and adversely affect the structure and function of microbial communities (Barbosa et al. 2008; Kang et al. 2008). The major source of environmental androgen contamination comes from human and animal excreta, similar to estrogen contamination (Lange et al. 2002; Lorenzen et al. 2004; Arnon et al. 2008). Increasing levels of androgen contamination in the aquatic environment have also been observed in recent years (Zheng et al. 2008; Sang et al. 2012; Fu et al. 2019) and have been detected in surface water, groundwater, rivers, and sediments (Zheng et al. 2008; Sang et al. 2012). Androgens promote a high proportion of males in some aquatic animals (Orn et al. 2006) and cause masculinization or virilization in females and reduce their reproductive capabilities (Orlando et al. 2004; Kang et al. 2008), which has consequential effects on aquatic ecosystem.

Several physical, biological, and chemical methods can be used to control estrogen and testosterone pollution. The most common methods used include photocatalytic degradation, advanced oxidation processes (AOPs), adsorption, and biological degradation or biotransformation (Zhang et al. 2015a). Among these methods, biological degradation is the most common, successful, and economical method. Some groups of bacteria and white-rot fungi can efficiently degrade or transform these pollutants in contaminated environments. These microbial systems use the estrogen and testosterone as carbon and energy sources, thereby degrading or transforming them into other less harmful or neutral compounds (Zhang et al. 2015b; Li et al. 2017). Studies have shown that the use of microbial consortium rather than a single pure culture is a more effective way of estrogen biotransformation (Johnson et al. 2014). Similarly, some microbial strains (bacterial and algal) in the environment can biotransform testosterone into other less-harmful by-products (Yang et al. 2010; Fu et al. 2019).

### Presence of steroid hormones

Natural and synthetic estrogens are generated from human and animal excretions, pharmaceutical, dairy, poultry, and meat industries (Adeel et al. 2017). Since the rise in global industrialization, the release of steroid estrogens in wastewater increases tremendously, which further raises the steroid estrogen level in various water bodies (Jiang et al. 2013). This enhanced steroid estrogen pollution became a major threat to aquaculture along with humans too. These estrogens can be detected in feces, liquid manure, solid waste collected from cattle, lagoon effluent, and in manure applied directly to agricultural land (Biswas et al. 2013). For these reasons, four of the most commonly found natural and synthetic steroid estrogens distributed in various parts of the earth include E1, E2, E3, and EE2. These estrogens are also referred to as micropollutants as they are present in minute quantities but can affect aquatic animals due to their continuous exposure

(Kim and Zoh 2016). The molar effective concentrations ( $EC_{50}$ ) and relative potency factor (RPF) with reference compound 17 $\beta$ -estradiol for E1, E2, E3, and EE2 are  $1.2e^{-12}$ ,  $1.8e^{-12}$ ,  $7.3e^{-12}$ , and  $1.0e^{-12}$  and 1.4, 1.0, 0.23, and 1.7, respectively (Bermudez et al. 2012; Conley et al. 2017). Various studies have confirmed the presence of these steroid estrogens globally in freshwater, groundwater, and wastewater. Conley et al. (2017) reported the occurrence and in vitro bioactivity of estrogen, androgen, and glucocorticoid compounds in stream water in the USA. Estrogens are released into the environment from animal excretions (Zheng et al. 2008), with pigs and poultry industry waste releasing about 58% E2 in feces, while 96% EE2 and 69% E3 are excreted through urine (Adeel et al. 2017). The distribution of E1, E2, E3, and EE2 and testosterone in the surface water of 12 different countries found in four subcontinents is shown in Table 2. Total estrogen concentration in surface water, suspended particulate matter, and sediments also vary with season. In the Yangtze estuary of China, the total estrogen concentration varies throughout the year, with the pattern of estrogen concentration in four successive seasons of the year being 3.92 to 14.54 ng/L in July, 3.22 to 16.36 ng/L in October, 10.42 to 20.61 ng/L in January, and 5.03 to 10.77 ng/L in May. Moreover, levels of synthetic estrogens were higher than natural estrogens throughout the year (Nie et al. 2015). The presence of steroid estrogens in groundwater in various countries has also been analyzed, with the results revealing that in China the concentrations of E3 and EE2 in groundwater varies between 0.03 and 0.09 ng/L (Li et al. 2013), whereas E1 and E2 were not detected (Chen et al. 2011). In European countries (France and Spain), the concentration of E1, E2, and EE2 in groundwater varied between 1.3 and 3.5 ng/L in France (Vulliet et al. 2008), with no estrogens detected in Spain's groundwater (Vulliet et al. 2008). In North America (USA and Mexico), no traces of estrogen were detected in Mexico groundwater (Felix-Canedo et al. 2013), but E1 (n.d.–79 ng/L), E2 (n.d.–147 ng/L), E3 (n.d.–1745 ng/L), and EE2 (n.d.–230 ng/L) were detected in the groundwater of USA (Karnjanapiboonwong et al. 2011). The presence of steroid estrogens in drinking water has also been evaluated, with the drinking water of mainland China reported to contain traces of E1 (n.d.–9.9 ng/L), E2 (n.d.–0.1 ng/L), and EE2 (n.d.–0.3 ng/L) (Fan et al. 2013; Zhang et al. 2011; Zhang et al. 2013). However, European (France, Italy, Spain) and American countries (Brazil and the USA) had no traces of estrogen in drinking water (Devier et al. 2013; Maggioni et al. 2013; Esteban et al. 2014b; Wang et al. 2011; Jardim et al. 2012; Falconer et al. 2006). In these studies, testosterone was only detected in China's (n.d.–480 ng/L) and Brazil's (n.d.–329 ng/L) groundwater and drinking water (Yang et al. 2014a; Montagner et al. 2019). Whereas in Japan, Malaysia, and Australia's groundwater and drinking water, its concentrations were below detection limits (Praveena et al. 2016). In Europe (France, Italy, Netherlands, Spain) and America

**Table 2** Geographical distribution of steroid estrogens and testosterone

Pollutants (ng/L)	Subcontinents													
	East Asia				Europe				America				Oceania	
Countries	China	Japan	Malaysia	France	Italy	Nether-land	Spain	Mexico	USA	Brazil	Argentina	Australia		
Estrone	n.d.–1.58 (Yang et al. (2014b))	17.1–107.6 (Furuichi et al. (2004))	n.d.–0.035 (Praveena et al. (2016))	0.8–3.9 (Cargouet et al. (2004))	n.d.–10 (Patrolecco et al. (2014))	n.d.–7.2 (Vethaak et al. (2005))	n.d.–17 (Esteban et al. (2014a))	n.d. (Felix-Canedo et al. (2013))	1.4–12.9 (Kolok et al. (2007))	n.d.–39 (Sodre et al. (2010))	n.d. (Valdes et al. (2015))	0.55–20.9 (Ying et al. (2009))		
Estradiol	n.d.–0.42 (Xu et al. (2014))	2.6–14.7 (Furuichi et al. (2004))	n.d.–0.0281 (Praveena et al. (2016))	0.8–3.6 (Cargouet et al. (2004))	n.d. (Patrolecco et al. (2014))	n.d.–1.0 (Vethaak et al. (2005))	n.d. (Esteban et al. (2014a))	n.d. (Felix-Canedo et al. (2013))	n.d.–8.8 (Kolok et al. (2007))	n.d.–7.3 (Sodre et al. (2010))	n.d.–369 (Valdes et al. (2015))	0.39–3.77 (Ying et al. (2009))		
Estriol	n.d.–0.37 (Xu et al. (2014))	n.d. (Furuichi et al. (2004))	n.d.–0.024 (Furuichi et al. (2004))	0.6–3.1 (Cargouet et al. (2004))	–	–	n.d. (Esteban et al. (2014a))	n.d. (Felix-Canedo et al. (2013))	n.d.–3.3 (Kolok et al. (2007))	n.d.–2.3 (Sodre et al. (2010))	n.d. (Valdes et al. (2015))	n.d.–1.9 (Ying et al. (2009))		
17 $\alpha$ -Ethinylestradiol	n.d.–3.43 (Yang et al. (2014b))	n.d. (Furuichi et al. (2004))	n.d.–3.29e <sup>-4</sup> (Praveena et al. (2016))	0.6–3.5 (Cargouet et al. (2004))	n.d. (Patrolecco et al. (2014))	n.d.–0.4 (Vethaak et al. (2005))	n.d. (Esteban et al. (2014a))	n.d. (Felix-Canedo et al. (2013))	n.d. (Kolok et al. (2007))	n.d.–25 (Sodre et al. (2010))	n.d.–43 (Valdes et al. (2015))	n.d.–0.52 (Ying et al. (2009))		
Testosterone/androgen	n.d.–480 (Yang et al. (2014a))	n.d.	n.d. (Praveena et al. (2016))	–	–	–	–	–	–	n.d.–329 (Montagner et al. (2019))	–	n.d.		

n.d., not detected

(Mexico, the USA, Argentina), no testosterone traces were found in groundwater and drinking water (Table 2).

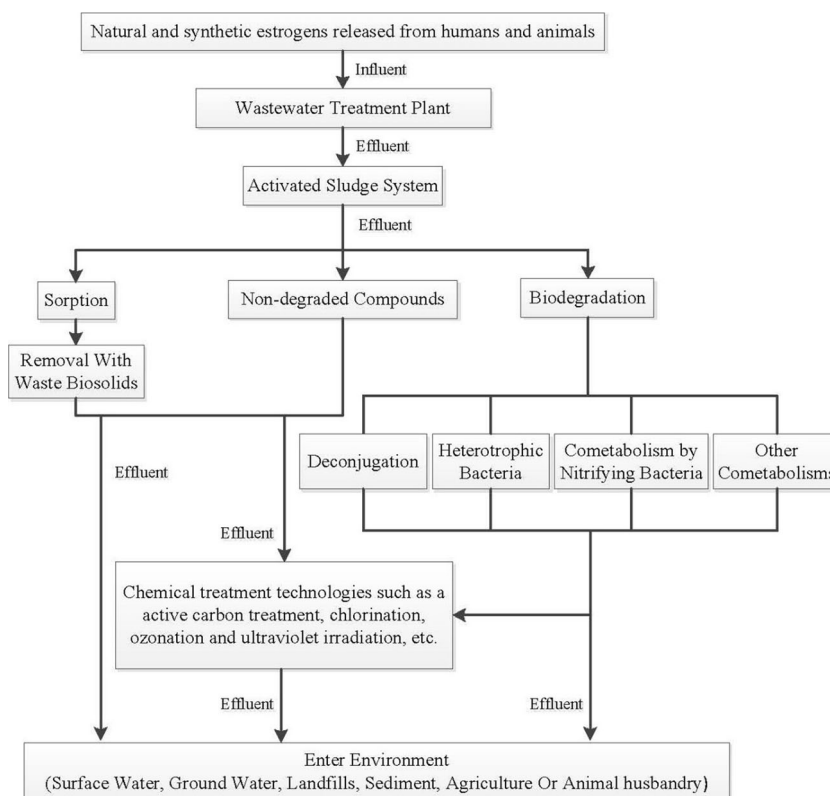
### Fate of steroid hormones

Steroid estrogens E1, E2, E3, and EE2 pollutants in wastewater are usually treated (biological or chemical) before releasing into the aquatic environment. Estrogens can be degraded by microbial communities present in soil and water under aerobic and anaerobic conditions. The half-life of estrogens varies with environmental conditions and concentration in a particular environment. The half-life of estrogens also depends on their degradation rate and oxygen availability (Adeel et al. 2017). Under aerobic conditions in soil, the half-lives of E1, E2, and E3 were found to be 2.8–4.9, 0.8–0.11, and 0.7–1.7 days, respectively (Biswas et al. 2013). Whereas in river water under aerobic conditions, the half-life of E1 and E2 were found to be 2–3 and 2–4 days, respectively (Jurgens et al. 2002; Ying et al. 2002; Adeel et al. 2017).

Liu et al. (2009b) determined the average concentration of steroid estrogens and natural androgens in municipal sewage wastewater treatment plants. The total excretion rate of estrogens E1, E2, and E3 was only 66–82% of total waste excretion. The concentration of these estrogens and natural androgen in urinary excretion varies with age and gender. For example, the estrogen excretion rates are different in premenopausal, pregnant, and postmenopausal women. The urinary excretion rate of E1, E2, and E3 for premenopausal, pregnant, and postmenopausal women were 10.73, 4.71, and 8.12, 1194, 347, and 24,078, and 5, 2.78, and 2.78  $\mu\text{g}/\text{day}$ , respectively, whereas for men it was 3.9, 1.5, and 1.5  $\mu\text{g}/\text{day}$ , respectively (Liu et al. 2009a). The urinary excretory rate of natural androgen (i.e., testosterone) for a man was 56.65 and 6.78  $\mu\text{g}/\text{day}$  for women (Liu et al. 2009a). Thus, the total concentration of steroid estrogens in sewage wastewater also depends on the gender ratio of that particular area. Estrogen removal during wastewater treatment is a well-practiced process and has been described by several research groups (Racz and Goel 2010; Xu et al. 2012; Luo et al. 2014; Liu et al. 2015). The diagrammatic illustration of estrogen's fate in a wastewater treatment plant is shown in Fig. 1. After mixing with activated sludge in the WWTPs, estrogens are either adsorbed onto solids in the wastewater-activated sludge or undergo biodegradation by various microbial systems (Racz and Goel 2010). Biodegradation is the major method used in estrogen removal from wastewater, which includes deconjugation, use of estrogen as carbon source by heterotrophic microorganisms, co-metabolism with nitrifying biomass, followed by another co-metabolism (Ke et al. 2007; Liu et al. 2016; Yu et al. 2016; Fernandez et al. 2017). There are variations in estrogen degradation or removal rate which mainly depend on different factors including operating conditions, geological location of the treatment plant, estrogen

concentration in effluent, and the type of biological system used (Yu et al. 2013). It has been reported that WWTPs equipped with biological treatment systems are much more effective in estrogen degradation as compared with those without biological treatments. More estrogen degradation has been observed in treatment plants that have long solid retention time (SRT) and long hydraulic retention time (HRT). Long SRT and HRT provide sufficient time for the growth of slow-growing estrogen degraders and longer contact time for estrogen-adsorbent materials (Maeng et al. 2013). Complete microbial degradation or biotransformation of steroids (estrogens and androgens) is challenging because of their complex chemical structure, low solubility in water, low number of functional groups, and the presence of four alicyclic rings, as well as two quaternary carbon atoms (Olivera and Luengo 2019). It has been shown that estrogens E1, E2, and EE2 removal ranges from 19 to 94, 76 to 92, and 83 to 87%, respectively in WWTPs (Baronti et al. 2000). The descending order of biodegradation rate of steroid estrogens in WWTPs are  $E3 > E2 > E1 > EE2$  (Garcia et al. 2019). In activated sludge, up to 98% E1 can be removed (Racz and Goel 2010; Zhou et al. 2012; Garcia et al. 2019), 99.9% E2 capable of being removed (Racz and Goel 2010; Heffron et al. 2016; Garcia et al. 2019), almost 100% E3 can be removed (Racz and Goel 2010; Garcia et al. 2019), while EE2 removal varies between 34% and almost 100% (Muller et al. 2008; Racz and Goel 2010; Garcia et al. 2019). Studies have also shown that estrogens concentration in effluent fluctuates daily, with no uniform or clear temporal trend despite consistency in wastewater flow (Williams et al. 2003; Racz and Goel 2010; Heffron et al. 2016). Even after activated sludge treatment, there is still a certain environmentally significant concentration of estrogens in the wastewater. For example, the biotransformation of E2 leads to the formation of E1. Generally, the microorganisms that biotransform E2 are unable to biotransform E1 further, which means that E1 removal from polluted water is relatively poor compared with E2 (Sami et al. 2006). It is partly, for this reason, that E1 starts to accumulate in water bodies and its concentration increase in the aquatic environment. Furthermore, it has been observed that E1 concentration in the surface water is much higher than other natural estrogens (Sami and Fatima 2019). In WWTPs, the fate of androgen can be tracked by metabolite analysis with isotope-labeled substrates. Under aerobic degradation of testosterone, most of the bacteria adopt 9,10-seco pathway. It has been shown that *Comamonas* spp. and *Pseudomonas* spp. are dominant in sewage that has testosterone traces. The meta-cleavage dioxygenase gene (*tesB*) of various proteobacteria is used to track this essential catabolic gene in sewage (Chen et al. 2016). In anaerobic degradation pathway, bacteria degrade androgens through the 2,3-seco pathway by the bifunctional 1-testosterone hydratase/dehydrogenase (Yang et al. 2016). Furthermore, to ensure efficient estrogen

**Fig. 1** Fate of steroid estrogens in wastewater treatment plants (modified from Racz and Goel 2010)



removal from wastewater, additional chemical treatment technologies such as activated carbon treatment, chlorination, ozonation, and ultraviolet irradiation, etc. are also carried out (Bila et al. 2007; Racz and Goel 2010; Hartmann et al. 2014; Li et al. 2016).

Studies using LC-MS/MS and GC-MS have revealed the presence of steroid estrogens E1, E2, and E3 and testosterone in WWTP-activated sludge along with their concentrations (ng/L) in influent, effluent as well as their removal percentage in five different countries, i.e., Japan, Italy, Australia, the USA, and Canada (see Table 3) (Liu et al. 2009b). Steroid estrogens and natural androgens are released into the environment as urinary (90–95%) and fecal (5–10%) waste from humans and animals. These estrogens are released into the environment in either conjugated or unconjugated forms, with most of the estrogen released with fecal matter being unconjugated, whereas estrogens released with urinary waste are mostly conjugated (Termes et al. 1999; Ascenzo et al. 2003; Liu et al. 2009a; Racz and Goel 2010). There are nine types of estrogen conjugates found in WWTP environment (Liu et al. 2009b). Recently, Yu et al. (2019) published a review on estrogen conjugates concentration (ng/L) present in various WWTP influents and effluents. The common estrogen conjugates along with their concentration (ng/L) in influent and effluent and their removal percentage in WWTPs are shown in Table 4. Data analysis showed that in WWTPs, the average removal rate of estriol, estradiol, and estrone conjugates are

90, 66, and 46%, respectively. Furthermore, it has been shown that the detection of any micropollutant in a medium depends on the limit of detection (LOD) or limit of quantification (LOQ) of that particular instrument. Thus, estrogen pollutants might not be detected in effluents (due to the detection range of the instrument), which does not mean that 100% of the steroid pollutants have been removed from the effluent after treatment (Naldi et al. 2016).

### Mechanism of steroid hormone biotransformation

Microorganisms can degrade or biotransform steroid hormones by three major mechanisms: (i) Growth linked (metabolic): where estrogens are used as the sole carbon and energy source by the microorganisms. (ii) Non-growth linked (co-metabolic): where microorganisms grow by using other carbon and energy sources found in the media and produce enzymes which catalyze estrogen biotransformation to form various products (Yu et al. 2013; Zhang et al. 2015b). Co-metabolism also refers to the simultaneous degradation of non-growth substrates by microorganisms using a nutrient substrate (Fernandez-Fontaina et al. 2014). Studies have shown that co-metabolism can initiate reactions, convert persistent compounds into potentially more biodegradable intermediates, and participate in central metabolic pathways for further biotransformation (Groning et al. 2007; Tran et al. 2013). Municipal wastewater contains numerous micropollutants such as antibiotics and estrogens but due to

**Table 3** Steroid estrogen and testosterone concentration (ng/L) in Influent and Effluent of activated sludge of WWTPs and their removal percentage (Liu et al. 2009a; b)

Name of compound	Country	Analysis method	Influent concentration (ng/L)	Effluent concentration (ng/L)	Removal (%)	Reference
Estrone	Japan	LC-MS/MS	259–326	n.d.–17	93–100	Kobayashi et al. (2006)
	Italy	LC-MS/MS	25–132	2.5–82	22–95	Baronti et al. (2000)
	Australia	LC-MS/MS	29–670	n.d.–72	111–100	Clara et al. (2005)
	USA	GC-MS	57.8–83.3	6.3–49.1	41–89	Robert et al. (2007)
	Canada	GC-MS	n.d.–33	n.d.–147	– <sup>a</sup>	Fernandez et al. (2007)
Estradiol	Japan	LC-MS/MS	n.d.–57	4.6–14 b	100–92	Kobayashi et al. (2006)
	Italy	LC-MS/MS	4–25	0.35–3.5	59–98	Baronti et al. (2000)
	Australia	LC-MS/MS	35–125	n.d.–30	44–100	Clara et al. (2005)
	USA	GC-MS	11.2–161.6	1.5–5.4	52–99	Robert et al. (2007)
	Canada	GC-MS	2.4–26	0.2–14.7	18.5–98.8	Servos et al. (2005)
Estriol	Japan	LC-MS/MS	n.d.	n.d.–151	100–0	Kobayashi et al. (2006)
	Italy	LC-MS/MS	24–188	0.43–18	77–99	Baronti et al. (2000)
	Australia	LC-MS/MS	23–660	n.d.–275	18–100	Clara et al. (2005)
	USA	GC-MS	79.7–259.2	2.2–3.9	95–98	Robert et al. (2007)
	Canada	GC-MS	n.d.–22	n.d.–29	– <sup>a</sup>	Fernandez et al. (2007)
Testosterone	Japan	LC-MS/MS	n.d.–11	n.d.	100	Liu et al. (2009b)
	USA	GC-MS/MS	– <sup>a</sup>	– <sup>a</sup>	– <sup>a</sup>	Kolodziej et al. (2009)
	Canada	GC-MS/MS	n.d.–95	n.d.–21	– <sup>a</sup>	Fernandez et al. (2007)

*n.d.*, not detected

<sup>a</sup>Data not available

their small quantity, they cannot support microbial growth but can induce microbial genes related to enzymes and cofactors which are involved in biodegradation (Fischer and Majewsky 2014). (iii) Convert steroid to metabolites but does not degrade the metabolites. Dehydrogenation of steroids is considered as a detoxication process (Hamid and Eskicioglu 2012). Most of the estrogen degradation is growth linked, where microorganisms use estrogen as a sole carbon source for their growth. Abiotic nitration or oxidation is also observed in some microbial systems. This mechanism is adopted by microorganisms in the presence of higher ammonia concentration (Yu et al. 2013). The rate of microbial degradation of estrone changes under the influence of background nitrogen and carbon. It has been seen that the use of ammonia as a nitrogen source enhances the estrone biotransformation rate significantly, probably because the presence of ammonia promotes tyrosine synthesis by promoting the GS-GOGAT pathway. The presence of acetic acid (AA) and humic acid (HA) also boost estrone biotransformation because the presence of these acids (AA and HA) triggers the up-regulation of tyrosine synthesis enzymes (Du et al. 2017). Besides, a higher concentration of ammonia also enhances EE2 biotransformation because ammonium oxidation provides more reducing power. Enhanced EE2 biotransformation has been observed in ammonia enriched sludge (Jantanprasartporn et al. 2017).

## Biotransformation of steroid estrogens

The complete degradation or transformation of steroids has been studied extensively due to their potential effects on the aquatic environment. The metabolic degradation of steroid estrogens is related to the degradation mechanism adopted by microorganisms. In this process, microorganisms use steroids as a sole carbon and energy source. This degradation mechanism is adopted only with the presence of high steroid concentration. Estrogens found in the environment can be degraded by both aerobic and anaerobic metabolic degradation pathways. The details of these (aerobic and anaerobic) microbial degradation pathways are given in subsequent sections.

## Aerobic microbial biotransformation

The aerobic microbial biotransformation of steroid estrogen is common in nature. It has been observed that aerobic degradation is faster in summers than in winters (Vieno et al. 2005). Aerobic biotransformation is carried out by bacterial, fungal, and algal species.

**Table 4** Estrogen conjugates of E1, E2, and E3 found in WWTPs (Yu et al. 2019)

Compound name	Conjugate name	Conc. in WWTPs influent (ng/L)	Conc. in WWTPs effluent (ng/L)	Removal percentage (average)	Reference		
Estrone	Estrone-3-sulfate	25	9	54.48%	Ascenzo et al. (2003)		
		42	13		Komori et al. (2004)		
		34.1	0.3		Reddy et al. (2005)		
		18	11.8		Kumar et al. (2009)		
		16.5	1.9		Zhu et al. (2015)		
		76	27		Naldi et al. (2016)		
		4.8	0.8		Ben et al. (2017)		
	4.4	0.7	Ben et al. (2018)				
	Estrone-3-glucuronide	4.3	0.7	35.7%	Ascenzo et al. (2003)		
		11	7.4		Komori et al. (2004)		
		0.4	n.d.–0.05		Reddy et al. (2005)		
		0.6	n.d.–0.6		Kumar et al. (2009)		
		3.6	n.d.		Zhu et al. (2015)		
		Estradiol	Estradiol-3-glucuronide		5.2	n.d.	99.9%
0.3					n.d.–0.07	Reddy et al. (2005)	
13.3	0.5			Zhu et al. (2015)			
2.0	0.4			Ben et al. (2017)			
2.5	0.7			Ben et al. (2018)			
Estradiol-17-glucuronide	18		91	40%	Komori et al. (2004)		
	n.d.–0.25		n.d.–0.25		Reddy et al. (2005)		
	10.5		n.d.–1.4		Zhu et al. (2015)		
	51		21		Naldi et al. (2016)		
	3		0.7		Ben et al. (2017)		
	3.6		0.9		Ben et al. (2018)		
	Estradiol-3-sulfate		3.3		n.d.	48%	Ascenzo et al. (2003)
110			52	Komori et al. (2004)			
32			n.d.–0.07	Reddy et al. (2005)			
3.6		0.4	Kumar et al. (2009)				
2.7		1.8	Zhu et al. (2015)				
13		5.3	Naldi et al. (2016)				
5.5		0.7	Ben et al. (2017)				
4.9		0.7	Ben et al. (2018)				
Estradiol-3,17-disulfate	n.d.–0.28	n.d.–0.28	78%	Reddy et al. (2005)			
	28	3.3		Naldi et al. (2016)			
Estriol	Estriol-3-glucuronide	22	72	n.d.	Komori et al. (2004)		
		n.d.–0.28	n.d.–0.8		Kumar et al. (2009)		
	Estriol-3-sulfate	14	2.2		84	Ascenzo et al. (2003)	
	Estriol-17-glucuronide	30	n.d.		100	Sueka et al. (2005)	

n.d., not detected

### Aerobic steroid biotransformation by bacteria

Steroid estrogen biotransformation by bacteria species is one of the most common and prominent ways of microbial

degradation. Over the last decade, a large number of steroids transforming bacteria species have been isolated from marine, river, compost, sludge, sandy aquifer (Ke et al. 2007), and soil samples. E2-biotransforming strains *Rhodococcus* sp. JX-2



and *Rhodococcus* sp. DS201 were mainly isolated from activated sludge samples (Liu et al. 2016; Yu et al. 2016). Both of these bacterial species effectively degrade 17 $\beta$ -estradiol (E2) from the environment. Another bacteria strain *Novosphingobium* sp. E2S with E2 transformation capability was also isolated from activated sludge samples. This strain transforms 66% of E2 after 7 days of incubation (Li et al. 2017). Due to the marine oligotrophic environment and high levels of salinity, strains that transform estrogen are difficult to isolate. So far, *Buttiauxella* sp. (Zhang et al. 2011), *Vibrio* sp. (Sang et al. 2012), *Rhodococcus* (Ye et al. 2017), *Virgibacillus*, and *Bacillus* (Fernandez et al. 2017) are the main estrogen-transforming strains isolated from the marine environment. Fernandez et al. (2017) isolated five 17 $\beta$ -estradiol anaerobic transforming bacterial strains (F1–F5) from deep-sea sediments.

### Aerobic steroid biotransformation by fungi

The non-bacterial microorganisms that are capable of transforming steroid estrogens are fungi. Several fungal species are reported to transform steroids. Mascoti et al. (2016) explored the biotransformation of dehydro-epi-androsterone in *Aspergillus parasiticus*. This fungal strain was able to effectively biotransform bicycle [3.2.0] hept-2-en-6-one, the standard Baeyer-Villiger monooxygenase (BVMO) substrate to produce testolactone and the homo-lactone 3 $\beta$ -hydroxy-17 $\alpha$ -oxa-D-homoandrost-5-en-17-one. Hunter et al. (2006) reported that *Aspergillus tamari* was a unique fungus that transforms progesterone into testolactone in high yield (about 70%) through a four-step enzymatic pathway, which is flexible for a range of steroidal substrates. A mycelium named *Curvularia lunata*, displayed a good capability of hydroxylation of steroids. This fungal strain transforms 16 $\Delta$ 5–3 $\beta$ hydroxy- and  $\Delta$ 4-3-ketosteroids of androstane and pregnane classes into 20 monohydroxy and dihydroxy-metabolites (Andrushina et al. 2011). Also, this fungus can dehydrogenate a wide range of different classes of steroids, because 17 $\beta$ -hydroxysteroid dehydrogenase from this strain (17 $\beta$ -HSDcl) could oxidize and reduce both estrogens and androgens, including estrone, 4-estrene-3,17-dione, 4-androstene-3,17-dione, and 5 $\alpha$ -androstane-3,17-dione (Lanisnik Rizner et al. 2001). The fungus *Fusarium moniliforme* can be used for transforming 3-hydroxy-steroids into their 7 $\alpha$ -hydroxylated derivatives (Cotillon and Morfin 1990). An inducible microsomal 7 $\beta$ -hydroxylase was characterized by this strain and was shown to be able to hydroxylate dehydroepiandrosterone (DHEA), pregnenolone, epiandrosterone, and estradiol at the 7 $\alpha$ -position. Furthermore, many other fungal strains that are capable of transforming steroids including *Fusarium oxysporum* var. cubense, which causes the Panama disease of bananas (*Musa* sp.) and *Exophiala jeanselmei* var. lecanii-cod, a

contaminant of the ginger plant (*Zingiber officinale*). These fungal strains are responsible for 7 $\alpha$  and 15 $\alpha$  hydroxylation of steroids and side-chain degradation as well as 1,2- and 1,4-reduction of steroidal enones, respectively (Wilson et al. 1999; Reese 2007).

### Aerobic steroid biotransformation by algae

Apart from bacterial and fungal strains, some algae can also efficiently transform steroid estrogens. The freshwater bacteria-free microalgae *Raphidocelis subcapitata* exhibits a strong ability to remove E2 and diethylstilbestrol (DES) by biotransformation (Liu et al. 2018b). Furthermore, the presence of DES enhances the removal of E2, which might be that DES stimulates enzymes such as glutathione *S*-transferase (GST), cytochrome P450, and peroxidase, which actively participate in E2 biotransformation (Shi et al. 2010; Peng et al. 2014; Gao and Chi 2015; Liu et al. 2018b). Wang et al. (2017) also reported an enhanced removal of EE2 by green microalgae in the presence of E2. Another freshwater alga, *Chlorella vulgaris*, can transform steroid estrogens present in sewage water. Steroid biotransformation rate depends on the concentration of algal cells (*C. vulgaris*) as well as the substrate concentration (Lai et al. 2002). Similarly, Shi et al. (2010) achieved efficient steroid estrogen transformation from pond water with the help of a complex inoculum of six different algal species along with duckweed. The six algal species, *Anabaena cylindrica*, *Chlorococcus*, *Spirulina platensis*, *Chlorella*, *Scenedesmus quadricauda*, and *Anaebena* var., were surprisingly able to biotransform E1, E2, and EE2, when present in nanogram concentrations (Shi et al. 2010). Besides, another algal species, *Scenedesmus dimorphus*, was able to efficiently biotransform steroid estrogens, with biotransformation efficiencies of about 85% for 17 $\alpha$ -estradiol and estrone and 95% for 17 $\beta$ -estradiol and estriol over 8 days (Zhang et al. 2014). Thus, based on present findings, the most general transformation processes of hazardous pollutants by microalgae, including hydroxylation, glycosylation, and methylation (Liu et al. 2018b). In general, the cytochrome P450 monooxygenase (CYP450) was found to be involved in the detoxification of steroid estrogens in microalgae (Torres et al. 2008).

### Anaerobic steroid microbial biotransformation

The biological transformation of steroids under anaerobic environment is considered recalcitrant (Czajka and Londry 2006). Since steroid biotransformation rate is much slower in an anoxic environment, these steroids become recalcitrant and start accumulating in sediments (Racz and Goel 2010). Also, the absence of active substances such as dissolved organic matter and Fe(III) in the anaerobic environment adversely affect the biotransformation rate of steroids, for which

reasons anaerobic microbial degradation of steroids is challenging (Gu et al. 2018). Very little is known about the anaerobic biotransformation mechanism of steroids. In the last two decades, only a few anaerobic steroids transforming microbial systems have been discovered (Dermer and Fuchs 2012). Anaerobic catabolism of steroids involves oxygen-independent steps that are well studied in the anaerobic breakdown of cholesterol. It has been discovered that anaerobic steroid transformation is biphasic, i.e., rapid and slow transformation phases, both of which can be described by first-order degradation kinetics (Zhang et al. 2015b). During anaerobic transformation, E2 is degraded much faster than E1 and EE2, while EE2 is almost non-biodegradable (Zhang et al. 2015a). Some research groups have successfully demonstrated the anaerobic transformation of testosterone (Chiang et al. 2010). It has been shown that anoxic transformation of steroids takes place during enterohepatic circulation in various mammals by intestinal bacteria. In this anoxic environment, the cleavage of alkyl aryl ether linkages, dehydroxylation, and oxidation or reduction at C-17 was found, but the breakdown of the steroid nucleus by these bacteria to obtain energy was not clearly described (Groh et al. 1993). In the denitrifying steps of wastewater treatment and anoxic river sediments, mineralization of estradiol has been observed but the exact mechanism of mineralization used by bacteria is still unclear (Fahrback 2006). Unfortunately, there is currently not much information on the mineralization of estrogens under anoxic conditions. However, the anaerobic transformation of E2 into E1 has been observed in acetone enriched activated sludge samples (Lee and Liu 2002). Besides, facultative anaerobes isolated from the deep sea (Fernandez et al. 2017) and sandy aquifer (Ke et al. 2007) could also transform E2 into E1 in an anaerobic medium. The list of some anaerobic microbial steroid degraders is given in Table 5.

### Anaerobic steroid transforming microbial consortia

Microbial consortia are a mixture of different microbial strains which are capable to degrade or transform a particular substrate present in the environment (Clark et al. 2009). In the consortium, more than one microbial strains are growing symbiotically and simultaneously participates in the transforming mechanism. The enrichment culture technique is considered as a powerful tool to obtain microbial consortia with desired degradation capabilities (Feng et al. 2011; Okeke and Lu 2011). Microbial consortia developed by this method are much closer to the consortia that are functioning in nature (Feng et al. 2011). In steroid-degrading consortia experiment, microbial consortia took from activated sludge. The microbial consortium were firstly enriched in mineral salt media spiked with steroids. After successful enrichment of these strains, species present in consortia were identified and characterized by using different molecular biology tools (Agarwal et al.

2015; Edet et al. 2017). Yu et al. (2005) first time used the quantitative fingerprinting method and the real-time-RFLP to determine three different microbial systems present in activated sludge samples which were responsible for the transformation of  $17\alpha$ -estradiol,  $17\beta$ -estradiol, and estrone. Secondly, Zanga et al. (2008) determined various phylogenic groups present in activated sludge samples by using micro autoradiography-fluorescence in situ hybridization (MR-FISH) technique. After that Real-time PCR assays were developed to determine estrogen degrading bacteria from consortia, this RT-PCR assay technique was further used to determine the average cell number of strain JEM-1 present in activated sludge (Hashimoto et al. 2010).

### Co-metabolic steroid transformation by nitrifying microorganisms

Most microbial systems use metabolic degradation pathways to degrade a particular substrate when its concentration is high in the environment. At lower substrate concentrations, the co-metabolic pathway is used by microorganisms to degrade/transform steroids. In co-metabolic pathways, steroids are transformed with the help of some enzymes released by the microbial system. In these cases, they are not used as a sole carbon and energy source. Thus, a primary growth substrate is required for sustainable microbial growth. Nitrifying bacteria can efficiently metabolize steroids by this pathway. The transformation of EE2 in nitrification tanks using ammonium monooxygenase (AMO) enzyme is an example of a co-metabolic transformation of steroids (Andersen et al. 2003). It has been proposed that the AMO gene product is responsible for steroid transformation under anoxic conditions, but it is still not clear that whether nitrifying bacteria or heterotrophic bacteria is responsible for steroid transformation (Kayee 2014). Various molecular biology techniques have been used to identify and isolate the genes involved in co-metabolic steroid transformation. Through this, several research groups have been able to successfully isolate, clone and express steroid transforming genes in suitable hosts to enhance the co-metabolic steroid transformation (Shao et al. 2016; Wang et al. 2017).

### Enzyme systems involved in estrogen and testosterone transformation

Microbial transformation of estrogen and testosterone is a complex physiological process that requires a series of catalytic reactions (Knol et al. 2008; Ye et al. 2017). The major enzymatic reactions involved in steroid transformation are hydroxylation (Van der Geize et al. 2008), isomerization (Talalay and Wang 1955), oxylation (Samavat and Kurzer 2015), acylation and hydrolysis. Major enzymes which catalyze these reactions are dehydrogenase (Genti-Raimondi et al. 1991; Ye

**Table 5** Some anaerobic steroid degraders isolated from various sources along with their degradation potential

Name of organism	Isolated from	Degradation potential	Reference
16S rRNA gene 95% similar to <i>Sphingomonas</i>	Sandy aquifer	Estrogen	Yu et al. (2016)
16S rRNA gene 99% similar to <i>Virgibacillus halotolerans</i>	Deep sea	17 $\beta$ -Estradiol	Fernandez et al. (2017)
16S rRNA gene 99% similar to <i>Bacillus flexus</i>	Deep sea	17 $\beta$ -Estradiol	Fernandez et al. (2017)
16S rRNA gene 99% similar to <i>Bacillus licheniformis</i>	Deep sea	17 $\beta$ -Estradiol	Fernandez et al. (2017)
<i>Denitratisoma oestradiolicum</i> AcBE2-1	Activated sludge	17 $\beta$ -Estradiol	Fahrbach (2006)
<i>Thauera</i> spp.	Anaerobic sewage	Androgen	Fujii et al. (2003)
<i>Alcaligenes</i> spp.	Deep soil sediment	Cholesterol	Ismail and Chiang (2011)
72Chol DSMZ 12783	Forest ditch mud	Cholesterol	Ismail and Chiang (2011)
<i>Sterolibacterium</i> ( <i>Sli.</i> ) <i>denitrificans</i> Chol-1ST DSMZ 13999	Forest ditch mud	Cholesterol	Ismail and Chiang (2011)
<i>Denitratisoma oestradiolicum</i> DSMZ 16959	Activated sludge	17 $\beta$ -Estradiol	Ismail and Chiang (2011)
<i>Steroidobacter denitrificans</i>	Anoxic digested sludge	17 $\beta$ -Estradiol, testosterone	Ismail and Chiang (2011)
Iron-reducing bacteria with 16S rRNA gene 84% similar to <i>Shewanella baltica</i>	Anaerobic digester	17 $\beta$ -Estradiol	Volodymyr et al. (2010)

et al. 2017), cytochrome P450 (Venkataraman et al. 2015), ring-cleavage dioxygenase, hydroxylase, monooxygenase, isomerase, hydratase, and demethylase (Kristan and Rizner 2012), etc. Also, it has been observed that various proteins such as electron transfer proteins, receptor proteins, signaling proteins as well as regulatory proteins are involved in the active transformation of steroid hormones (Penning 2003; Miller and Auchus 2011; Xu et al. 2017; Acconcia and Marino 2018). The most important enzymes that regulate steroid hormone biotransformation are hydroxylase and dioxygenase.

### Short-chain dehydrogenase

The short-chain dehydrogenase/reductase (SDR) is a large enzyme family that is primarily involved in the metabolism of various hormones such as steroids, retinoids, etc. Some essential enzymes found in human beings of this family are epimerase, hydratase and NAD (P)H-dependent oxidoreductase (Simard et al. 1995). Most of the proteins of this family are made up of 250–300 amino acid residues and mostly consist of at least 2 domains, which first binds to coenzyme and the second binds to the substrate. The common microbial enzymes involved in steroid degradation from this family are HSD i.e., 17 $\beta$ -hydroxysteroid dehydrogenase and 3 $\alpha$ -hydroxysteroid dehydrogenase. HSD has been characterized as the main or the sole enzyme that initiates the catabolism of cholesterol or other sterols such as estradiol in *Mycobacteria*, *Nocardia*, and *Rhodococcus* sp. (Wipperman et al. 2014; Kreit 2017; Ye et al. 2017).

### 17 $\beta$ -Hydroxysteroid dehydrogenase

3 $\beta$ , 17 $\beta$ -hydroxysteroid dehydrogenase (3 $\beta$ , 17 $\beta$ -HSD, EC 1.1.1.51) is directly related to steroid metabolism. The first

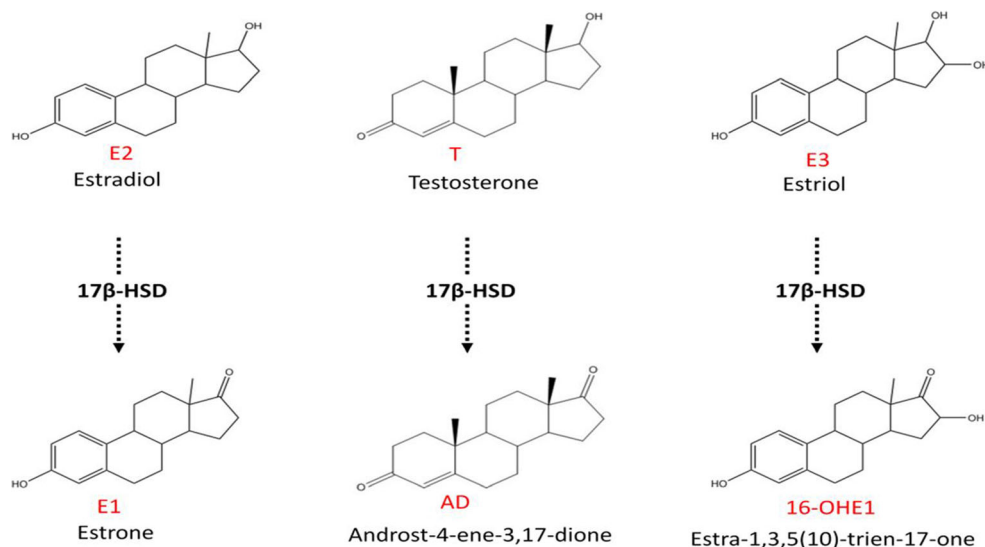
step in steroid transformation, i.e., conversion of estradiol to estrone (Fernand et al. 1997) was catalyzed by this enzyme (Fig. 2). Studies have revealed that 17 $\beta$ -HSD plays a major role in the conversion of the active form of steroid hormones into non-active forms. In filamentous fungus, 17 $\beta$ -HSDcl from *Cochliobolus lunatus* can catalyze the same reactions similar to some human enzymes (Rizner et al. 1996).

Cassetta et al. (2005) demonstrated that the Tyr167 amino acid residue was an active center of fungal 17 $\beta$ -HSDcl. Besides, 17 $\beta$ -HSD plays an active role in *Comamonas testosterone* and causes the complete oxidative degradation of steroid skeleton (Yu et al. 2015). Furthermore, Ye et al. (2017) also described the presence of 17 $\beta$ -HSD homologs in *Rhodococcus* sp. P14, which effectively converted toxic estradiol into less toxic estrone. In *Pseudomonas putida* SJTE-1, two genes (*crgA* and *oxyR*) adjacent to 17 $\beta$ -HSD encoded the potential *CrgA* and *OxyR* regulators, which are under the regulation of the 17 $\beta$ -HSD gene. *CrgA* could enhance the transcription of 17 $\beta$ -HSD, while *oxyR* represses 17 $\beta$ -HSD expression (Fernand et al. 1997).

### 3 $\alpha$ -Hydroxysteroid dehydrogenase

3 $\alpha$ -hydroxysteroid dehydrogenase (3 $\alpha$ -HSD, E.C. 1.1.1.50) acts on various steroid hormones by catalyzing the redox reaction of the hydroxylatone group at position 3 of C19–27 on steroids. The strain *C. testosteroni* (*Pseudomonas testis*) expresses this dehydrogenase (Oppermann et al. 1993; Abalain et al. 1995), which can reduce the ketone group present at position 3 of testosterone. Also, this strain expresses the isoenzyme 3 $\alpha$ -hydroxysteroid dehydrogenase/carbonyl reductase (3 $\alpha$ -HSD/CR) (M.W. 49.4 kDa), which further reduces the toxicity of testosterone (Maser et al. 2000). X-ray

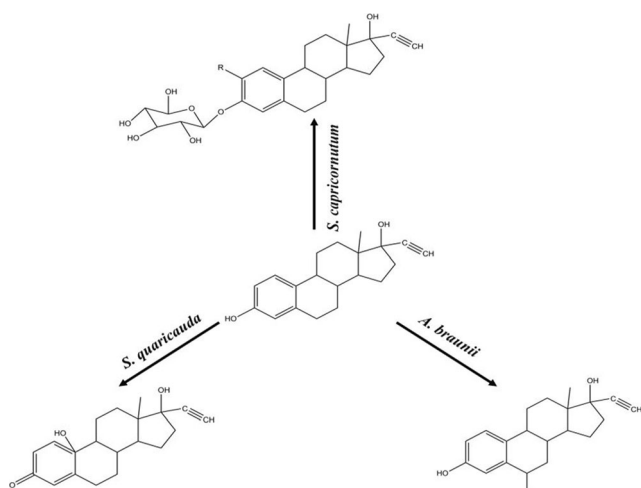
**Fig. 2** Dehydrogenation of common estrogen with  $17\beta$ -HSD enzyme (modified from Ye et al. 2019)



crystallographic studies revealed that each asymmetric unit of the enzyme is a homodimer in nature.

## Hydroxylase

Bacterial hydroxylase is one of the important components of the steroid catabolic pathway and plays an important role in steroid transformation. These enzymes help in steroid breakdown by opening the B-ring. Hydroxylation of ethinylestradiol by strain *Selenastrum capricornutum*, *Scenedesmus quadricauda*, and *Ankistrodesmus braunii* is shown in Fig. 3 (Greca et al. 2008). The most common hydroxylases involved in steroid transformation are Rieske-type non-heme iron oxygenase and Cytochrome P450.



**Fig. 3** Hydroxylation of Ethinylestradiol by strain *Selenastrum capricornutum*, *Scenedesmus quadricauda* and *Ankistrodesmus braunii* (modified from Greca et al. 2008)

## Rieske-type non-heme iron oxygenase

These types of enzymes (3-ketosteroid  $9\alpha$ -hydroxylase (KSH)) are involved in the 9,10-seco pathway of testosterone biotransformation. This is the only known core aerobic pathway of steroid transformation by bacteria (Horinouchi et al. 2012). The opening of the steroid structure is initiated by the alpha-hydroxylation of C9 position. Microbial species such as *Nocardia* (Strijewskim 1982), *Rhodococcus* sp. (Knol et al. 2008), and *Mycobacterium neoaurum* (Yao et al. 2014) express KSH enzymes. These KSH enzymes are mainly of two types, i.e., oxygenase (KshA) and flavin-dependent ferredoxin reductase (KshB), both of which are required for the KSH activity (Van der Geize et al. 2008; Petrusma et al. 2009). Studies have revealed that KSH works along-side 3-anthrone- $\Delta$ 1-dehydrogenase (KstD) to form  $9\alpha$ -hydroxyandrost-1,4-diene-3,17-dione ( $9\alpha$ -OH-ADD), where KSH is responsible for cleaving the CC bond at positions 9 and 10 or AD alone which produces  $9\alpha$ -hydroxyandrost-4-ene-3,17-dione ( $9\alpha$ -OH-AD) (Yao et al. 2014).

## Cytochrome P450

Cytochrome P450 (CYP) is a class of heme and thiol-rich proteins which is widely distributed among living organisms. On the bases of their involvement in electron transport during a catalytic reaction, cytochrome P450 can be categorized into four different categories: the first type is a FAD-containing reductase and iron-sulfur protein. This is the most abundant type of CYP and so far most of the bacterial enzymes found to belong to this class. The second type of enzymes requires P450 reductase containing FAD and FMN, which are electron transport systems found in microsomes. The third type of enzyme does not require any electron donor, whereas, in the

fourth type of enzyme, electrons are acquired directly from NAD(P)H (Urlacher and Girhard 2012).

In prokaryotic organisms, cytochrome P450 is a soluble protein found in the cytosol whereas in eukaryotic organisms it is membrane-bound. Cytochrome P450 is a typical heme protein that catalyzes the hydroxylation of aromatic and aliphatic substrates (Bernhardt and Urlacher 2014). Monooxygenase cytochrome P450 was discovered for the first time to be involved in aerobic hydroxylation of steroid hormones in 1963 (Cooper et al. 1963), while the first bacterial cytochrome P450 was discovered in *Bacillus megaterium* in the mid-1970s (Berg et al. 1975). A limited number of bacteria cytochrome P450 involved in estrogen conversion have been discovered. The commonly found CYP450 are *Bacillus megaterium* (Berg et al. 1975; Kille et al. 2011; Schmitz et al. 2014; Jozwik et al. 2016), *Nocardia farcinica* IFM 10152 (Bracco et al. 2013), and *Streptomyces griseus* (Makino et al. 2014).

Structural studies of the cytochrome P450 families revealed that the primary structure has fewer similarities but the spatial structure of all P450 enzymes belonging to various families are highly conserved and have higher similarities with each other (Tsuchiya et al. 2005; Nelson 2005). Usually, a typical cytochrome P450 structure contains 13 different  $\alpha$ -helices (A–L) and four  $\beta$ -sheet domains from the N-terminus to the C-terminus, which form an inverted triangle structure. Detailed studies of cytochrome P450 showed that its complex protease system requires electron transport chains in addition to the terminal oxygenases. The conserved three-dimensional structure of cytochrome P450 seems to have some connection with its catalytic activity for various organic pollutants (Szaleniec et al. 2018).

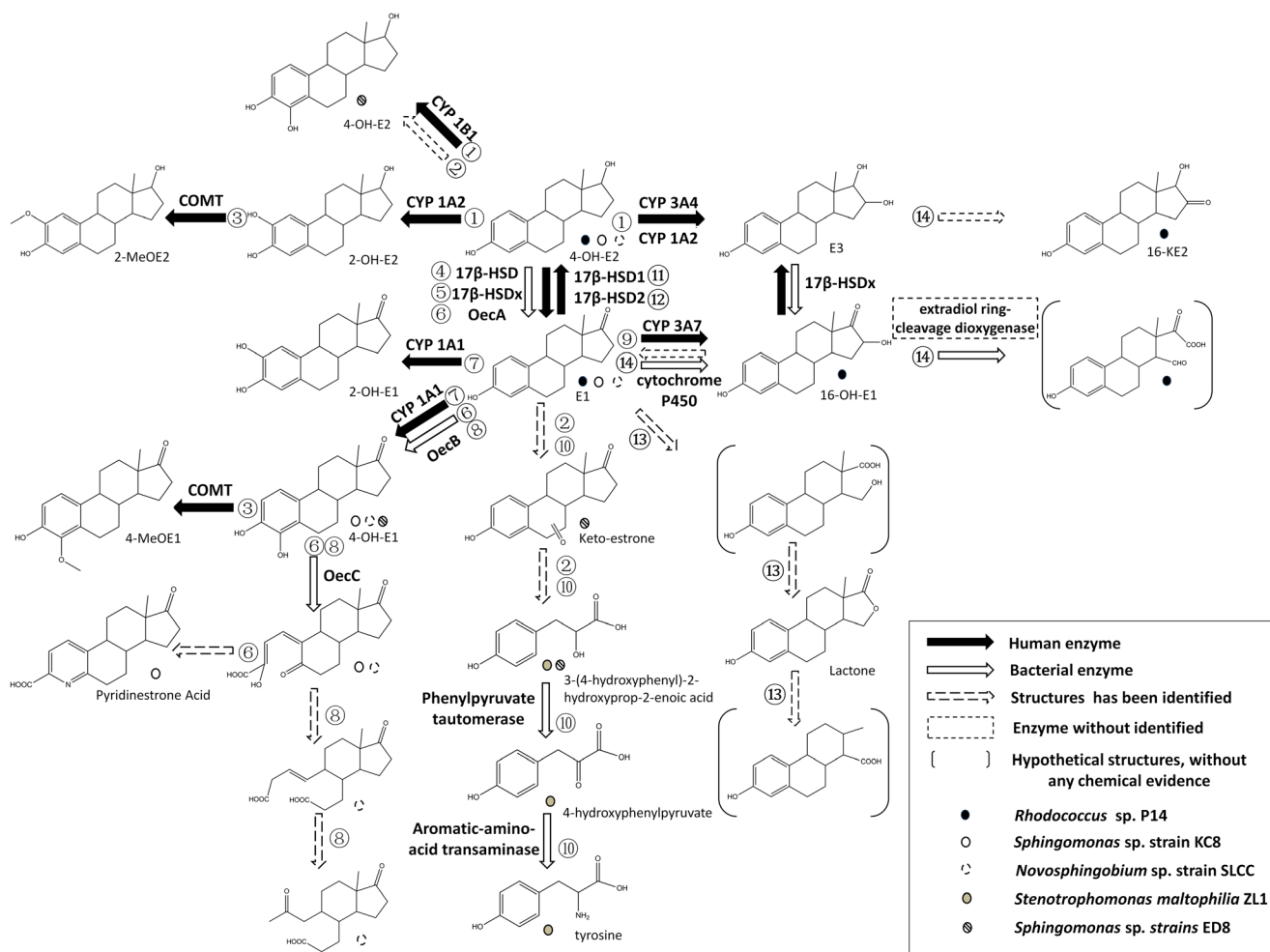
### Ring cleavage dioxygenase

Biological transformation of aromatic compounds such as steroids is mostly initiated by the enzyme ring-hydroxylating dioxygenase, which converts them into diol intermediate compounds (Martin and Mohn 1999; Shindo et al. 2007). Further, ring cleavage dioxygenase enzyme could cleave the A ring/B ring of these diol intermediate compounds (Horinouchi et al. 2012; Chen et al. 2018). Various studies concluded that different microbial species followed different steroid metabolizing pathways, but these all metabolizing pathways produce some common intermediates such as protocatechuic acid, catechol (Guevara et al. 2019), gentisic acid, hydroquinone, propionyl-CoA (Liu et al. 2018a), acetyl-CoA (Xu et al. 2017), tyrosine (Li et al. 2012a; b), etc. The cyclic cleavage dioxygenase acts on these common intermediate metabolites and opens their benzene rings. Chen et al. (2017) described for the first time, the role of enzymes which actively participated in the ring cleavage of estrogens. The initial reaction of the estrogen catabolic pathway by strain

KC8 was demonstrated. It was demonstrated that E1 transformation is initiated with the oxygenolytic degradation of the aromatic A ring through the 4-hydroxylation and the subsequent meta-cleavage reactions. It is well known that E2 is being transformed into E1 with the help of 17 $\beta$ -estradiol dehydrogenase enzyme, which is the product of *OecA*. Furthermore, this E1 was transformed into 4-hydroxyestrone in the presence of estrone 4-hydroxylase enzyme which is the product of *OecB* (Gene cluster I). In strain KC8, *OecC* was found up-regulated which indicates that *OecC* plays an important role in estrogen catabolism. This *OecC* belongs to the type I estradiol dioxygenase family, which uses ferrous ion to catalyze meta-cleavage of catechols and their analogs, while under abiotic conditions it leads to the production of pyridinestrone acid. The details of the enzymatic estrogen ring cleavage are shown in Fig. 4 (Chen et al. 2017). Also, aromatization of A-ring of testosterone can be carried out by a meta-cleavage enzyme (Horinouchi et al. 2012). Especially, *tesB* from *Comamonas testosteroni* TA441 is necessary for testosterone degradation (Horinouchi et al. 2001).

### Summary of microbial enzymes involved in estrogen biotransformation

Estrogen biotransformation is a broad topic, with studies on estrogen-metabolizing enzymes mainly carried out in humans. Studies on bacterial estrogen-transforming enzymes were initially focused on dehydrogenation reactions (Ye et al. 2017; Ye et al. 2019). Chen et al. (2017) proposed a complete microbial degradation metabolic pathway of estradiol in *Sphingomonas* sp. strain KC8, by detecting intermediate components, genomic analysis, transcriptome analysis and other techniques to study the enzyme involved in various reactions. In strain KC8, 4,5-seco pathway, an aerobic estrogen catabolic pathway having three estrogen catabolic gene clusters, i.e., *oecA*; KC8\_09390, *oecB*; KC8\_16650, and *oecC*; KC8\_05325 was described. These three gene clusters were responsible for the expression of enzymes 3 $\beta$ , 17 $\beta$ -hydroxysteroid dehydrogenase, estrone 4-hydroxylase, and 4-hydroxyestrone 4, 5-dioxygenase, respectively, which were directly involved in the meta-cleavage of estrogen A ring (Chen et al. 2017). Besides, Lee et al. (2007) also tried to detect the metabolic pathway of estradiol degradation in *Stenotrophomonas maltophilia* ZL1 by using genomic and proteomic analysis techniques and revealed the up-regulation of aromatic-amino-acid transaminase. Extensive studies on 17 $\beta$ -estradiol transforming enzymes (both microbial and human) has been carried out in previous couple of years which resulted the characterization or identification of several new intermediate compounds during these biotransformation reactions (Greca et al. 2008; Urlacher and Girhard 2012; Bracco et al. 2013; Bernhardt and Urlacher 2014; Ye et al. 2017; Ye et al. 2019). A



**Fig. 4** Over all compilation of estrogen degradation by various microbial enzymes. (References: ①, Badawi et al. (2001); ②, Kurisu et al. (2010); ③, Tsuchiya et al. (2005); ④, Ye et al. (2017); ⑤, Ye et al. (2019); ⑥, Chen et al. (2017); ⑦, Kisselev et al. (2005); ⑧, Chen et al. (2018); ⑨,

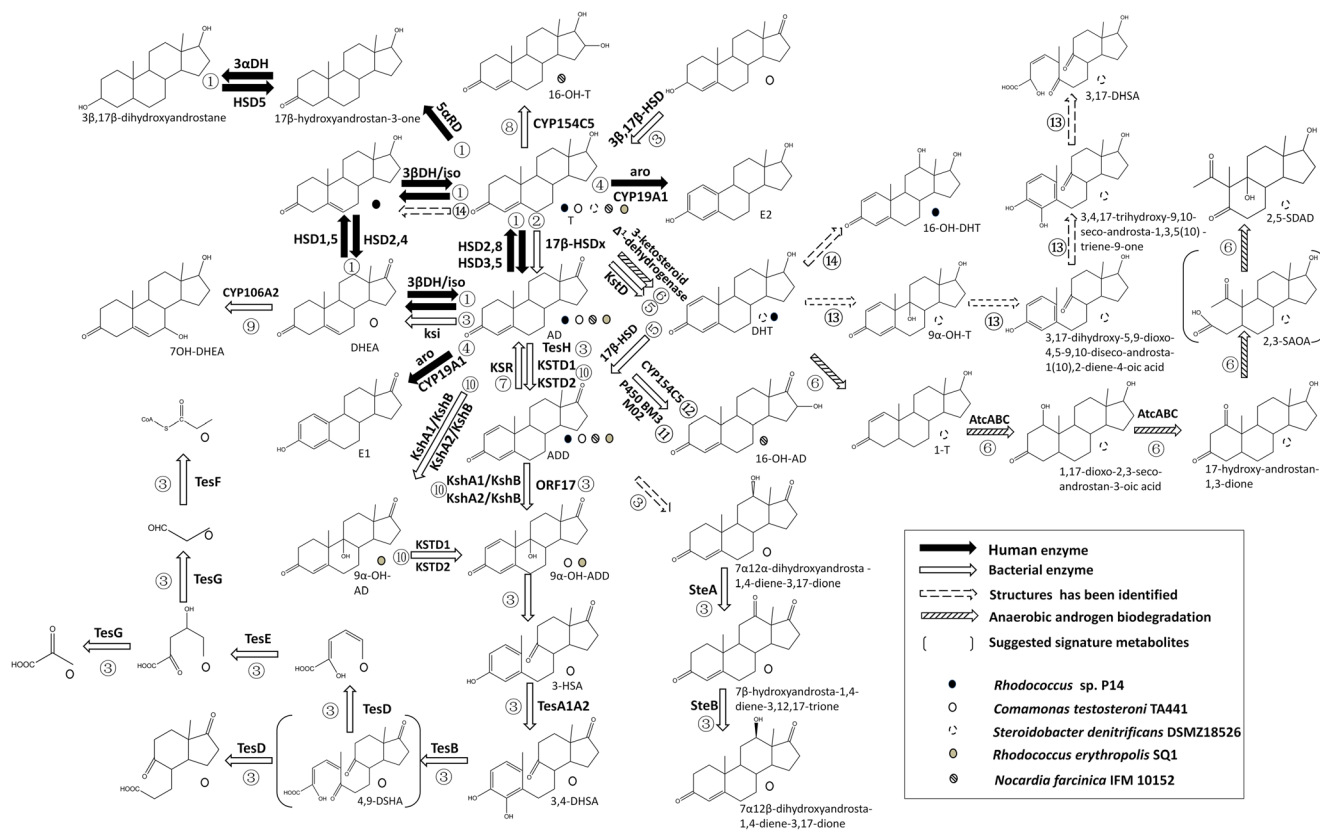
Lee et al. (2003); ⑩, Li et al. (2012a, b); ⑪, Breton et al. 1996; ⑫, Moeller and Adamski (2006); ⑬, Lee and Liu (2002); ⑭, Ye et al. (unpublished work))

combined diagrammatic representation of all intermediates formed during  $17\beta$ -estradiol transformation by both human and microbial enzymes is compiled in Fig. 4.

### Summary of microbial enzymes involved in testosterone biotransformation

Microbial biotransformation of testosterone has been one of the hot research topics for the last two decades, and a large number of research articles have been published on this topic. The most common microbial species that are capable of transforming testosterone are *Steroidobacter denitrificans* DSMZ18526 (Fahrbach et al. 2008; Chiang et al. 2010; Wang et al. 2014), *Rhodococcus erythropolis* SQ1 (Knol et al. 2008; Van der Geize et al. 2008), *Nocardia farcinica* IFM 10152 (Ishikawa et al. 2004; Bracco et al. 2013), and *Comamonas testosteroni* TA441 (Horinouchi et al. 2003; Horinouchi et al. 2012). The *Comamonas testosteroni* TA441 is a classical organism that has several enzymes that

aerobically transform testosterone (Fig. 5). The production of androstenedione (AD) by  $17\beta$ -hydroxysteroid dehydrogenase, followed by  $\Delta 1$ -dehydrogenase ( $\Delta 1$ -dehydrogenase, TesH), which is further converted to androst-1,4-diene-3,17-dione (ADD). The ADD is responsible for the introduction of hydroxyl group at C-9 through ORF17, which causes  $9\alpha$ -hydroxylation (the process causes the generation of a very unstable intermediate), at the same time the B-ring cleavages take place accompanied by aromatization of the A-ring. This process generates 3-hydroxy-9,10-diol-1,3,5(10)-triene-9,17-dione (3-hydroxy-9,10-seco-androsta-1,3,5(10)-triene-9,17-dione (3-HSA)) as a product. Furthermore, TesA1A2 enzyme complex hydroxylate C-4 cleaves the core ring and produces 4-dihydroxy-9,10-nonanediol-1,3,5(10)-triene-9,17-dione (3,4-dihydroxy-9,10-seco-androst-1,3,5(10)-triene-9,17-dione (3,4-DHSA)). Next, TesB Bio-oxygenase splits the A ring to 4,5-9,10-diseco-3-hydroxy-5,9,17-trioxoandrost-1(10),2-dien-4-oic acid (4,9-DSHA). Finally, after multiple steps, the testosterone is mineralized into carbon dioxide and



**Fig. 5** Over all degradation of androgen along with their intermediates by various microbial enzymes. (References: ①, Mindnich et al. (2004); ②, Ye et al. (2019); ③, Horinouchi et al. (2012); ④, Yoshimoto and Guengerich (2014); ⑤, Chen et al. (2012); ⑥, Yang et al. (2016); ⑦,

Vanden et al. 1993); ⑧, Badawi et al. (2001); ⑨, Schmitz et al. (2014); ⑩, Van der Geize et al. (2008); ⑪, Venkataraman et al. (2015); ⑫, Bracco et al. (2013); ⑬, Wang et al. (2013); ⑭, Ye et al. (Unpublished work))

water. It shows that the *TesB* gene cluster includes 18 different types of androgen transforming genes (Horinouchi et al. 2003). This cluster is widely found in androgen transforming bacterial species such as *Burkholderia*, *Comamonas*, *Cupriavidus*, *Glaciecol*, *Hydrocarboniphaga*, *Marinobacterium*, *Novosphingobium*, *Pseudoalteromonas*, *Pseudomonas*, *Shewanella*, and *Sphingomonas* (Chen et al. 2016; Olivera et al. 2018). This method of testosterone metabolism by the aerobic bacterial system is known as the 9-10-seco pathway.

In addition to the aerobic 9-10-seco pathway, an anaerobic catabolic pathway was also observed in *Steroidobacter denitrificans* DSMZ18526. This pathway is different from the aerobic pathway. In this transformation route, under controlled conditions, testosterone was first converted into 1-dehydrotestosterone by the action of 3-ketosteroid $\Delta$ 1-dehydrogenase. This first step is the divergent step between the aerobic and anaerobic pathways of testosterone transformation. Under anoxic conditions, the catabolic process starts from ring A. This bacterial species encodes the gene of the molybdenum protein of the xanthine oxidase family, *AtacABC* (anaerobic testosterone catabolism), which introduces a hydroxyl group at C-1 and oxidizes the C-1 hydroxyl group to form 17-hydroxyandrostane-1,3-dione. Phylogenetic analysis of this gene

indicates that the enzyme belongs to the family of xanthine oxidases containing molybdenum, FAD, and iron clusters (Yang et al. 2016). Besides, studies revealed that anaerobic biotransformation of testosterone was also carried out by 2,3-seco pathway (Yang et al. 2016). For instance, CYP154C5 from *N. farcinica* could catalyze T into 16 $\alpha$ -OH-T (Bracco et al. 2013) and DHEA could be catalyzed by CYP106A2 to form 7 $\beta$ -OH-DHEA (Schmitz et al. 2014). Interestingly, androgens could be converted to estrogens by the steroid aromatase, CYP19A1 (Yoshimoto and Guengerich 2014). Androgen metabolism by various microbial enzymes and their intermediate compounds (identified and unidentified) are shown in Fig. 5 (Talalay and Wang 1955; Nobel et al. 2001; Morris et al. 2003; Penning 2003; Horinouchi et al. 2003; Ishikawa et al. 2004; Fahrbach et al. 2008; Van der Geize et al. 2008; Chiang et al. 2010; Horinouchi et al. 2012; Bracco et al. 2013; Wang et al. 2014; Chen et al. 2016; Yang et al. 2016; Ye et al. 2017; Ye et al. 2019).

### Genetic adaptation mediated steroid biotransformation

Microbial systems are designed by nature to be adaptable, as bacterial cells can adapt to different habitats, including

contaminated and extreme environments and still be capable of performing various physiological activities (Dash et al. 2013). It has been observed that when some microbial strains are subjected to the high-stress environments (PAH and steroid pollution), they adapt to that environment and start growing by using the stress elements as energy and carbon source (Haritash and Kaushik 2009; Wang et al. 2018). This is termed adaptation, as pre-exposure to a high dose of contaminants increases the potential of the microbial community to metabolize or oxidize pollutants (Wang et al. 2018). Several studies have reported that adaptation increases the rate of organic pollutant transformation (Bergstrand et al. 2016). The genetic adaptation process that might lead to the induction and repression of enzymes, genetic changes, and selective enrichment has been defined as three mechanisms for adaptation of microbial communities to chemical contaminants (Leahy and Colwell 1990). The primary mechanism for detecting genetic adaptation capability of the microbial community is the identification and amplification of genes that are directly or indirectly involved in the metabolism of the organic contaminant by selective enrichment and gene transfer. The monitoring and identification of adaptation to steroid contamination are made possible by the generation of DNA probes specific for the genes encoding steroid catabolic pathways. Furthermore, the identification of genetic adaptation can be carried out by comparative genomic analysis (Bergstrand et al. 2016).

### Current state of steroid biotransformation

The above discussion shows that steroid pollution is harmful to aquatic animals and aquatic ecosystem as these pollutants have potential effects on sexual development and, the reproduction process in these animals. As society develops, there is a corresponding increase in steroid pollution. Presently, several microbiological research groups all over the world are working on the mechanisms of steroid biotransformation.

In the last couple of years, a significant number of research papers describing the microbial transformation of estradiol, estriol, and 17 $\alpha$ -ethinylestradiol have been published. Li et al. (2012a, b) reported the transformation of E2 into E1 by strain *Stenotrophomonas maltophilia* ZL1; proteomic data analysis revealed that strain *S. maltophilia* ZL1 can convert E1 to amino acid tyrosine through ring cleavage on a saturated ring of the E1 molecule and further use this tyrosine in protein biosynthesis. They also observed that the presence of the environmental tyrosine could affect the biotransformation pathway of E2/E1 by causing the feedback inhibition process. Besides, Yoneda et al. (2016) compared the 33rd and 40th passage generations of *Rhodococcus opacus* PD630 by using comparative genomics and transcriptomics methods and then identified an up-regulation in the expression of degradation genes, which helped to clarify the action of phenol transporter genes. The evolutionary relationship between these two

strains was analyzed, with the comparative genomics data revealing that the use of toxic compounds as sole carbon source accelerated the accumulation of single nucleotide polymorphisms (SNPs), which further caused targeted mutations. Using various techniques to decipher information on estrogen-degrading genes and degradation products helps to understand the biodegradation of organic pollutants. Further, the strain *Pseudomonas putida* Pf5 was used for estradiol degradation and the expression of functional proteins was analyzed using iTRAQ (Du et al. 2017). The results revealed that various factors such as stress responses, energy metabolism, transport, chemotaxis, cell motility and changes in protein production by carbon metabolism, especially over-expression of carbon-metabolizing proteins lead to the activation of nucleotide metabolic pathways along with carbohydrate pathways, which are mainly associated with energy metabolism (Du et al. 2017). For further investigation, environmental estrogen biotransformation kinetics was analyzed by quantitative proteomics analysis of *Hydrogenophaga atypica* ZD1 under various nitrogen and carbon backgrounds. It was observed that branched-chain amino acid aminotransferase (IlvE) might play an important role in E1 biodegradation (Xu et al. 2017). Similarly, transcriptomics and lipidomics analysis of *R. rubber* strain, an efficient polyethylene degrader, showed that this strain can change its membrane composition to control the rate of polyethylene transformation (Gravouil et al. 2017). Chen et al. (2017) identified and validated three estrogen catabolic gene clusters in *Sphingomonas* sp. KC8 strain and also successfully demonstrated its natural estrogen-transforming activity by steroid 4,5-seco pathway. To investigate sterol biotransformation in wild-type *Mycobacterium neoaurum* ATCC 25795, Liu et al. (2018a) used integrated transcriptomics and proteomics to identify the key genes and key metabolic activities of mutant strains producing steroid intermediates. Our group also reported a novel 17 $\beta$ -hydroxysteroid dehydrogenase (17 $\beta$ -HSD) enzyme from *Rhodococcus* sp. P14, which is capable of transforming E2 into E1. This enzyme (17 $\beta$ -HSD) is for the first time, reported in bacterial species which can oxidize E2 into E1 (Ye et al. 2017). As revealed in most of the reports, microbial systems can transform estradiol, estriol, and 17 $\alpha$ -ethinylestradiol into estrone. Estrone is less harmful than other steroids, due to its low concentration in nature. Most of the steroids are being transformed into E1, which ultimately raises E1 concentration in the environment. The higher concentration of E1 also has endocrine-disrupting effects on aquatic animals. Unfortunately, very few studies have reported on or explained E1 biodegradation. Most of the E1 degradation reports are associated with some physical degradation techniques. Recently, our group reported a new microbial strain that is capable of biotransforming E1, along with this transformation process, two new non-accumulating intermediate metabolites, i.e., 3-hydroxyandrost-5,7,9(11)-trien-17-one and androst-1,4,6-triene-3,17-dione (ATD) were also



characterized (Pratush et al. 2019). We hope to intensify our research to identify and characterize more E1-transforming microbial systems that could be used to remediate E1 concentration in aquatic systems.

## Conclusion and future perspective

In this review, we collected various information on the generation of steroids, their entry into the environment, and their effects on aquatic animals as well as on humans. In recent years, the scientific community is very much aware of steroid pollution. This review highlights the widespread contamination of the aquatic environment by different types of steroid estrogens. In the past few years, several steroid-transforming microorganisms have been isolated from soil, water, sediments, and marine water. These microorganisms are believed to transform steroids by metabolic and co-metabolic pathways, while the mechanisms involved are not clear, hence there is a need for further research on this. It has been also observed that the WWTPs are unable to degrade estrogens without biological treatment systems, for which reason these steroids find their way into water bodies. On the other hand, the WWTPs equipped with a biological treatment system also has some limitations, as the microbial systems have very low transforming capability; therefore, these systems need long SRT and HRT, which increase the load on these treatment setups. To overcome these problems, microbial systems with high and fast degrading/transforming capabilities, which are also environmentally friendly, would have to be engineered or generated. Research should also be focused on degradation/ transformation kinetics to improve overall steroid metabolism. Also, modern techniques such as multi-omics analysis, isotope labeling would have to be used to detect new products. Presently, a large number of oxic (aerobic) microbial steroid transforming systems have been isolated from different sources. The oxic steroid transformation found to be much more efficient than anoxic transformation. However, only very few anoxic microbial species are identified that could effectively transform steroid estrogens. Thus, for effective estrogen transformation in anoxic environments, new anoxic microbial species must be isolated. Furthermore, studies on anoxic metabolism of steroids by bacteria should address the following issues: (i) whether the cleavage of the core ring system of steroid compounds begins from the A-ring, (ii) whether the elimination of C17-alkyl side chain precedes cleavage of the core ring system in the anoxic cholesterol catabolic pathway, (iii) if a common pathway for steroid metabolism by denitrifying bacteria is potentially

involved in both oxic and anoxic conditions, and (iv) the purification and characterization of novel steroid-transforming enzymes (Ismail and Chiang 2011). The researcher would also have to focus their work on finding facultative anaerobic microbial strains, which can transform steroids in both the presence and absence of oxygen. For both microbial transformations (aerobic and anaerobic), many challenges still exist in steroid biotransformation. The major challenge is the low biotransformation productivity of microbial strains, which is not economically viable to use for industrial applications. The other problems that need to be solved include low steroid solubility, insufficient substrate availability, and in some cases, the toxicity of the substrate/product to microbial cells. Apart from scientific efforts, strict regulations must be put in place to check steroid pollution.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

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