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REVIEW

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# Biodegradation of conjugated estrogens in wastewater treatment: species, mechanisms, and influencing factors

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**Abstract**

Estrogenic pollutants, especially conjugated estrogens (CEs), have become an important environmental problem due to their potential interference with aquatic ecosystems and threats to human health. As an effective means, biodegradation plays a key role in the removal of CEs in natural and artificial systems. After long-term adaptation and domestication, the bacterial species that can tolerate and degrade CEs were retained, thereby achieving efficient removal of CEs. In this paper, the important role of bacteria in the biodegradation of CEs was reviewed. The bacterial species and pure isolates related to the biodegradation of CEs were described in detail. The mechanism of enzymatic hydrolysis and the subsequent degradation process of free estrogen were discussed. The biodegradation products and possible degradation pathways of CEs were summarized. In addition, the effects of environmental factors on the biodegradation efficiency of CEs were also discussed. This paper aims to summarize the research progress of CE biodegradation and put forward future research prospects.

**Keywords** Conjugated estrogens, Bacteria, Enzymatic hydrolysis, Biodegradation, Wastewater treatment

## Introduction

The issue of environmental hormones has garnered global attention, particularly steroidal estrogens (SEs), which were of particular concern to the public and scientific community [1–3]. Estrogens are found in the environment from various sources, including natural metabolites and those synthesized by human activities [4–9]. Based on their chemical form, estrogens can be classified as free estrogens (FEs) and conjugated estrogens (CEs), and their properties are closely related to the specific structure of the phenolic and alcoholic hydroxyl groups [10–14]. The hydroxyl group of FEs is replaced by an ester bond, a structural change that masks their endocrine-disrupting effects until hydrolysis occurs [15–17]. CEs, when hydrolyzed to their free form, can exhibit increased estrogenic activity by $10^2$ to $10^5$ times, significantly increasing the risk of endocrine disruption [18–20]. CEs can enter aquatic environments through wastewater treatment plants (WWTPs) and release FEs. Even at concentrations below 0.1 ng/L, FEs can cause significant estrogenic effects in the aquatic environment [21–23]. Therefore, further in-depth research and assessment of the potential impact and effective treatment methods for these contaminants are necessary.

The ubiquitous presence of CE pollution and increasing regulatory pressures have driven the demand for efficient CE treatment technologies. In water or soil matrices, some physical and chemical treatment methods, such as adsorption and advanced oxidation, have proven to be effective [24–28]. However, compared to these methods, microbial-mediated biodegradation of CEs has attracted significant attention because of its low cost and

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environmental friendliness [29–32]. Biodegradation has been confirmed as an effective method for dealing with estrogenic pollutants, such as bisphenol A, estradiol, and estrone [27, 33–39]. With the development of genetic sequencing technology, researchers have identified certain bacteria from the environment capable of degrading CEs and have obtained genomic information on these strains [40–42]. These advances have made it possible to further explore the biodegradation mechanisms of CEs.

They exhibit significant potential for environmental remediation mainly because of their ability to produce a variety of enzymes, including hydrolases, peroxidases, oxidases, and redox enzymes [36, 43]. These enzymes can decompose macromolecular polymers into smaller monomers [44], which can be used as carbon and energy sources by microorganisms, and ultimately achieve the mineralization of pollutants [36]. Moreover, the biodegradation process may also produce by-products, which may be more susceptible to natural degradation [36, 40]. Nevertheless, there was a scarcity of comprehensive reviews that delve into the biodegradation of CEs, emphasizing the pressing need for an in-depth examination. Such a review would elucidate the most recent advancements in the biodegradation of CEs, offering theoretical insights and a reference framework for their management in wastewater systems. This paper presents, for the first time, a synthesis of the biodegradation byproducts and potential pathways of CEs. Additionally, it explores the influence of environmental factors, including temperature and dissolved oxygen levels, on the efficiency of CE biodegradation.

## Characterization of conjugated estrogens in WWTP

SEs, both CEs and FEs, possess a steroidal structure similar to that of cholesterol, featuring four carbon rings consisting of an aromatic ring (A ring), two hexane rings (B and C rings), and a pentane ring (D ring). The structures of different estrogens are identical in terms of their steroidal frameworks, but they vary in their functional groups [16, 39]. CEs are formed by the attachment of glucuronic acid or sulfate groups to the hydroxyl groups of FEs, resulting in G-CEs or S-CEs, with the chemical bonds being glycosidic and sulfate ester bonds, respectively [16]. The most studied CEs are those formed with a hydroxyl group at the 3rd position, such as estrone-3-glucuronide (E1-3G), estrone-3-sulfate (E1-3S), estradiol-3-glucuronide (E2-3G), estradiol-3-sulfate (E2-3S), estriol-3-glucuronide (E3-3G), estriol-3-sulfate (E3-3S), ethinylestradiol-3-glucuronide (EE2-3G), and ethinylestradiol-3-sulfate (EE2-3S), with some research also focusing on CEs at the 17 (16) position of E2, such as estradiol-17-glucuronide (E2-17G) [19, 23, 28, 31, 43, 45–48]. The physicochemical properties, together with the structure of common estrogens, are shown in Table 1.

During the wastewater treatment process, CEs can dissociate, leading to the release of FEs. Moreover, the concentration of CEs in wastewater is at the nanograms per liter (ng/L) level, which necessitates detection methods with higher sensitivity and precision. With the rapid development of detection instruments and techniques, it is now possible to directly detect and quantify CEs [49, 50]. Currently, there is a certain research foundation for determining CEs using chromatography-mass spectrometry methods (Table 2). For instance, ultra-high-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) combines chromatography with mass spectrometry, enabling the accurate and sensitive quantification of target compounds [51–55].

As both a source and sink of steroid estrogens, WWTPs play a significant role in the management of estrogen pollution [53, 56, 57]. SEs are primarily present as FEs and S-CEs in the influent of wastewater treatment plants, with the prevalence of sulfate ester conjugates reaching 100%. The median concentrations for these conjugates are 4.8 ng/L for E1-3S, 5.5 ng/L for E2-3S, and 14.7 ng/L for E3-3S [58–60]. In contrast, the glucuronide conjugates exhibited a lower frequency of detection and median concentrations, with values of 4.2 ng/L for E1-3G, 2.0 ng/L for E2-3G, and 3.0 ng/L for E2-17G [19, 61]. This lower prevalence may be attributed to the increased likelihood of these conjugates dissociating into their free forms via the enzymatic action of β-glucuronidase during their transit within the sewer system [62].

## Biodegradation mechanism of ces

The biodegradation of CEs is primarily constrained by the adsorption process, which involves the transfer from the aqueous phase to the sludge phase [65]. This process can be effectively characterized by the Freundlich model, illustrating the adsorptive capacity of activated sludge for CEs (Eq. (1)) [66].

$$q_e = \frac{x}{m} = K_F C_e^{1/n} \tag{1}$$

where $q_e$ is amount of the substance adsorbed on sludge at adsorption equilibrium ($mg{\cdot}g{\cdot}TSS^{-1}$), $x$ is the amount of substance adsorbed ($g{\cdot}m^{-3}$), $m$ is the amount of adsorbent ($g{\cdot}L^{-1}$), $K_F$ ($mg^{1-1/n}{\cdot}(m^3)^{1/n}{\cdot}g{\cdot}TSS^{-1}$) and $n$ are constants depending on temperature, the adsorbent, and the substance to be adsorbed. The adsorption characteristics were described by means of the determination of characteristic values and by the establishment of isotherms.

Under neutral conditions, there is a robust linear correlation between the logarithmic value of the octanol–water partition coefficient ($LogK_{ow}$) of CEs and their adsorption affinity for activated sludge [65]. Typically, the adsorptive capacity of activated sludge for CEs is less than that for

**Table 1** The properties and concentrations of CEs in wastewater

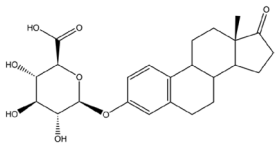
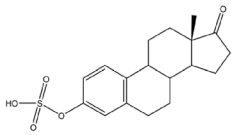
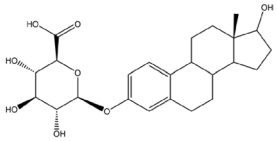
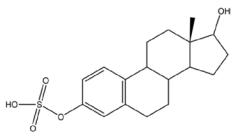
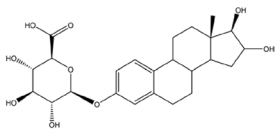
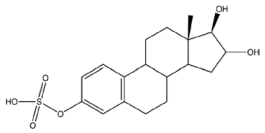
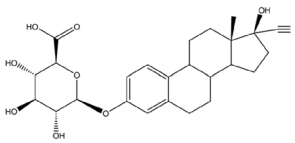
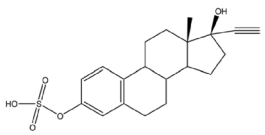
| CEs | MW (g/mol) | $LogK_{ow}$ | Structure | Influent (ng/L) | Effluent (ng/L) | Reference |
|---|---|---|---|---|---|---|
| E1-3G | 446.48 | 1.58 |  | 0.4–6.5 | N.D–3.0 | [19, 28, 31, 51, 52, 63] |
| E1-3S | 350.43 | 0.95 |  | 4.4–160.0 | 0.3–27.0 | [19, 28, 31, 51, 52, 63] |
| E2-3G | 448.50 | 2.10 |  | 0.3–13.3 | N.D–12.0 | [52, 53, 63] |
| E2-3S | 352.44 | 1.46 |  | 0.5–79.0 | N.D–141.0 | [54, 57, 61, 63] |
| E3-3G | 464.50 | 0.56 |  | ND–19.0 | N.D–72 | [55, 60, 61] |
| E3-3S | 368.44 | 0.32 |  | 1.4–44.1 | 0.1–6.5 | [19, 49, 51] |
| EE2-3G | 472.52 | 2.27 |  | 15.3–50.1 | 0.23–5.9 | [28] |
| EE2-3S | 376.46 | 1.63 |  | 0.17–0.7 | N.D–0.14 | [28] |

**Table 2** Quantitative detection method of CEs

| Compounds | Instrument | LOQ[a] (ng/L) | RE[b] (%) | RSD[c] (%) | Reference |
|---|---|---|---|---|---|
| E1-3S, E2-3S, E3-3S, E1-3G, E2-3G, E3-3G | UPLC-MS/MS | 0.07–1.29 | 81.0–116.1 | 0.6–13.6 | [46] |
| E1-3S, E2-3S, E3-3S, E1-3G, E2-3G, E3-3G | GC–MS | 5.40–6.80 | 73.3–114.9 | 1.6–19.9 | [64] |
| E1-3S, E2-3S, E3-3S | GC–MS | – | 64.0–112.3 | 3.6–18.4 | [50] |
| E2-17A, E1-3S, E2-3S E1-3G, E2-17G | LC–MS/MS | 1.0–30.0 | 23.0–87.0 | 2.0–9.0 | [54] |
| EE2-3S, EE2-3G | UPLC-MS/MS | 0.29–3.23 | 80.7–117.8 | 1.6–18.8 | [28] |
| E1-3S, E2-3S, E3-3S E1-3G, E2-3G, E2-17G | HPLC–MS/MS | 0.04–1.40 | 61.2–122.4 | 0.6–8.0 | [19] |

[a] Limit of quantifications

[b] Recovery efficiencies

[c] Relative standard deviations

their corresponding FEs, attributed to the lower hydrophobicity of CEs [66].

The biodegradation mechanisms of CEs involve two critical steps (Fig. 1): deconjugation facilitated by enzymatic hydrolysis, and the subsequent biodegradation of liberated free estrogens [67, 68]. These enzymes, including arylsulfatase and β-glucuronidase produced by bacteria, act under specific environmental conditions to cleave the ester bonds of estrogen conjugates, thereby releasing FEs into the aqueous phase [68–73]. Arylsulfatases are a class of enzymes that can break down aromatic sulfate esters into aromatic compounds and inorganic sulfates [74]. The hydrolysis reaction is represented by Eq. (2). Aromatic compounds such as E2 can continue to be biodegraded into smaller organic molecules until completely mineralized. In particular, in an anaerobic environment, the sulfate produced by the hydrolysis of CEs by arylsulfatase may be utilized by sulfate-reducing microorganisms, while the aromatic compounds themselves or the smaller organic molecules generated from biodegradation may serve as electron donors, thereby triggering the dissimilatory sulfate reduction process [75–79].

$$\mathrm{R\text{-}O\text{-}SO_3^- + H_2O \rightarrow R\text{-}OH + H^+ + SO_4^{2-}} \quad (2)$$

β-glucuronidase is an exoglycosidase that cleaves the glucuronic acid–O bond, breaking down glucuronide into the monosaccharide D-glucuronic acid and the corresponding aglycone [80]. Both arylsulfatases and β-glucuronidases have been detected in wastewater and activated sludge. However, the activities of these enzymes in activated sludge are much higher than those in wastewater. Specifically, the activity of β-glucuronidase in activated sludge is 18–68 times that in raw wastewater, while the activity of arylsulfatase is 1196–2776 times higher [31]. Although the activity of β-glucuronidase in raw wastewater was significantly higher than that of arylsulfatase, the difference in activity between these two enzymes in activated sludge was not significant. Moreover, whether in wastewater or activated sludge, the G-CEs of estrogens can be completely hydrolyzed within 24 h [19, 81–83]. Although the activity of arylsulfatase in raw wastewater is relatively low (only 4.2 U/L, it is not sufficient to significantly hydrolyze estrogen sulfates within 24 h; the conditions in activated sludge can achieve this process within 12 h [31, 43, 73]. This indicates that activated sludge is an important site for S-CE removal.

The deconjugation efficiency of activated sludge toward CEs is predictable, and by utilizing the rate constants presented in Table 3 and the model described by Eq. (3) [39], the timeframe for deconjugation within the activated sludge can be projected.

$$\ln\frac{A}{A_0} = -k_{dec} \times \mathrm{MLSS} \times t \quad (3)$$

Here, A and $A_0$ are the concentrations of estrogen conjugates at time $t$ and zero, $t$, $A$, MLSS, and $k_{\mathrm{dec}}$ denote the reaction time (hours), mixed liquor suspended solid concentration (gSS/L), and deconjugation rate constant (L/gSS·h), respectively.

In the context of wastewater treatment facilities, the overall removal efficiency ($R$) of FEs, is commonly calculated using the formula provided in Eq. (4) [39].

$$R = \frac{E_{in} - E_{out}}{E_{in}} \times 100\% \quad (4)$$

where $E_{in}$ and $E_{out}$ represent the concentrations of natural estrogens in the influent and effluent, respectively. The FEs released post-deconjugation, such as E1, E2, and E3, subsequently undergo biodegradation processes.

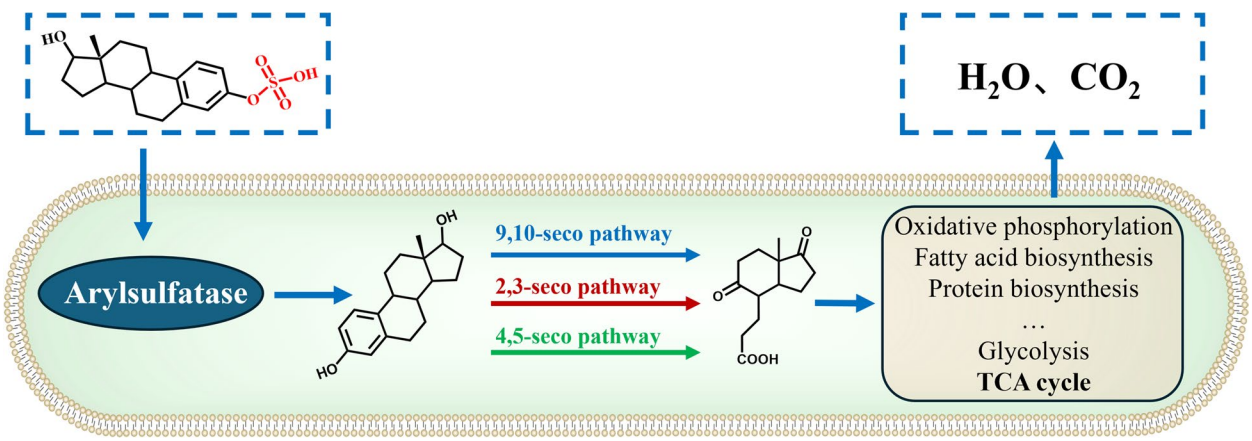



**Fig. 1** Biodegradation processes of CEs (exemplified by E2-3S) [84, 85]

**Table 3** Estrogen conjugate conjugation rate constants

| Target | Operation | pH | T(℃) | $C_i$[a](ng/L) | MLSS (g/L) | $k_{bio}$[b] (L/gSS/h) | Reference |
|---|---|---|---|---|---|---|---|
| E1–3S | Aerobic | 7.1 | 17 | 2348 | 4 | 0.00319 | [34, 62] |
| E1–3G | Aerobic | 7.1 | 17 | 2517 | 4 | 0.0875 | [39, 62] |
| E3–16G | Aerobic | 7.1 | 17 | 2323 | 4 | 0.06 | [39, 62] |
| E2–3S | Aerobic | – | – | 5E4 | 4–12.5 | 0.0728–0.13 | [39, 48] |
| E3–3G | Aerobic | – | – | 5E4 | 4–12.5 | 0.26–0.42 | [39, 48] |

[a] Target compound concentration

[b] Biodegradation rate constant

Therefore, if CEs also exist, *R* should be calculated using Eq. (5) [39]

$$R = \frac{E_{in} - E_{out} + C_{in} - C_{out}}{E_{in} + C_{in} - C_{out}} \times 100\% \quad (5)$$

where $C_{in}$ and $C_{out}$ refer to the concentrations of FEs derived from CEs.

## Key microbial species and metabolic mechanisms involved in the degradation of ces

As the understanding of the environmental impact of CEs deepens, researchers are committed to exploring effective biodegradation methods. An increasing number of bacteria in activated sludge, biofilms, and river sediments have been identified and isolated. Concurrently, with the advancement of biological sequencing and testing technologies, the biodegradation mechanisms and transformation pathways of CEs are gradually being refined.

### Major bacteria involved in the degradation of ces

Within the intricate matrices of activated sludge, biofilms, and riverine sediments, a diverse array of bacterial strains with an exceptional capacity to degrade CEs has been identified [86–88]. Through rigorous analysis of 16S rRNA gene sequences, these bacterial isolates have been meticulously classified at the species level, encompassing a spectrum of genera such as *Rhodococcus, Pseudomonas*, and *Alcaligenes* [44, 89, 90]. A particularly significant degrader is *Escherichia coli* (*E. coli*), which possesses a remarkable ability to secrete enzymes, including β-glucuronidase and arylsulfatase, which are pivotal for the hydrolysis of glucuronide- and sulfate-conjugated estrogens, respectively [59, 91–93]. In untreated domestic wastewater, the β-glucuronidase produced by *E. coli* demonstrates exceptional efficacy, facilitating the deconjugation of glucuronide-conjugated estrogens with notable ease [94–96]. Members of genera such as *Streptomyces*, *Microbacterium*, and *Rhodococcus* have been identified as prolific secretors of arylsulfatase enzymes, which hydrolyze sulfate-type conjugated estrogens [44]. This enzymatic process not only aids in environmental detoxification but also fulfills the microbial requirement for inorganic sulfate, a crucial component of metabolic processes [97, 98]. In *Streptomyces* sp., sulfatase activity is observed in two distinct forms: one integrated within the cell membrane and another intracellularly localized. Intracellular sulfatase is specifically induced under conditions of inorganic sulfate scarcity, whereas membrane-bound sulfatase appears to be triggered by substrate presence or is regulated independently of sulfur demands [44]. *Microbacterium* and *Rhodococcus* bacteria display a pronounced predominance of sulfatase activity within their cell membranes, constituting an impressive 98.75% of the total enzymatic activity [44]. In contrast, minimal sulfatase activity was detected within the intracellular and extracellular compartments, suggesting a specialized adaptation for the degradation of conjugated estrogens [44]. Genomic DNA analysis of these three bacterial strains revealed candidate sequences associated with sulfatase activity, which exhibited a high degree of homology with known members of the sulfatase gene family. This finding suggests the presence of at least two distinct arylsulfatase genes within the genomes of *Streptomyces* sp., *Microbacterium sp*., and *Rhodococcus* sp., with one gene presumed to be intracellular and the other likely associated with membrane structure, underscoring the complex interplay between these bacteria and their environmental niche [44].

In addition to the aforementioned genera, bacteria isolated from river sediments have also demonstrated proficiency in degrading estrogen conjugates. Species such as *Pseudomonas* sp., *Rhizobium* sp., and *Acinetobacter* sp. have been recognized as active contributors to this environmental detoxification process [67, 99]. The ability of diverse bacterial strains to degrade conjugated estrogens is a testament to their metabolic versatility and highlights their potential for bioremediation (Table 4). The metabolic capabilities of these bacteria represent a significant area of interest for environmental microbiologists and

**Table 4** Confirmed CEs degrading functional bacteria

| Type of enzyme | Genus | Species | Reference |
|---|---|---|---|
| Arylsulfatase | *Pseudomonas* | *Pseudomonas aeruginosa* | [72, 99] |
| Arylsulfatase | *Rhodococcus* | *Rhodococcus* sp. | [44, 74] |
| Arylsulfatase<br>β-glucuronidases | *Escherichia Castellani and Chalmers* | *Escherichia coli* | [20, 59] |
| Arylsulfatase | *Streptomyces* | *Streptomyces griseorubiginosus* | [44, 74] |
| Arylsulfatase | *Microbacteriaceae* | *Microbacterium* sp. | [100] |
| Arylsulfatase | *Acinetobacter* | *Acinetobacter calcoaceticus* | [101] |
| β-glucuronidases | *Clostridium* | *Clostridium* sp. | [102] |

biotechnologists, offering insights into the vast potential of harnessing microbial communities for biodegradation of complex organic pollutants. Understanding the genetic and enzymatic mechanisms that drive these degradation processes is essential for devising targeted strategies for augmenting the natural detoxification capabilities of these ecosystems. As research in this domain continues to advance, it is expected that these bacterial strains will play an increasingly pivotal role in the development of next-generation bioremediation technologies, thereby contributing to the realization of a more sustainable and healthier environment.

### Microbial metabolism of glucuronide-conjugated estrogens

Current research on the enzymatic degradation and transformation of G-CEs predominantly focuses on the glucuronide forms of E1 and E2, while relatively few studies have been conducted on the conjugated form of E3 [64, 100, 103, 104]. The biodegradation of G-CEs relies on β-glucuronidase, which belongs to the glycoside hydrolase class. This enzyme is produced by bacteria, fungi, higher plants, and animals, and is widely present in sewage, activated sludge, soil, and sediments [67, 101, 102]. E1-3G is completely cleaved to E1 under the action of β-glucuronidase and is subsequently utilized by microorganisms to achieve complete mineralization [105]. In contrast, the degradation pathway of E2-3G is more complex. The degradation of E2-3G follows a simple first-order kinetic model with rate constants ($k$) and half-lives ($t_{1/2}$) of 0.0355 and 18 h, respectively [20]. In environments where β-glucuronidase is abundant, E2-3G is primarily cleaved at the C3 position to form E2, which can be further oxidized to E1 [48, 106]. In conditions where β-glucuronidase is less available, oxidation at the C17 position can also occur, leading to the formation of E1-3G [16, 20]. The maximum percentage conversion of E2-3G to E2 was 34% (equivalent to a molar percentage of 56%), and after 4–9 days, approximately 20% of E2 was converted to E1 [20]. In addition to the aforementioned common metabolites, microbial degradation of E2-3G produces four novel products (metabolites I, II, III, and IV). Metabolites II, III, and IV have been identified as 9,11-dehydro-E1,6-keto-E1, and estrone, respectively, while the structure of metabolite I remains unclear and may correspond to keto-E2 or hydroxy-substituted E1 [20, 107–110]. Figure 2 shows the preliminary pathways of conjugated compound degradation. The degradation of E2-3G primarily involves the oxidation of hydrolysis products E2 and/or E1, followed by hydroxylation, desaturation, and lactonization to form hydroxy-E1, 9,11-dehydro-E1, and estrolactone; additionally, E2 and E1 can be further transformed into Keto-E1 [111–113]. Hydroxy-E1 is a pivotal intermediate in the estrogen degradation pathway [114]. It undergoes oxidative cleavage via the 4,5-seco pathway, leading to the formation of HIP [40, 84]. Eventually, portions of these compounds are further mineralized into $CO_2$.

### Microbial metabolism of sulfate-conjugated estrogens

Under both aerobic and anaerobic conditions, the biotransformation pathways of sulfate-conjugated estrogens exhibit significant differences, particularly for 17α-estradiol-3-sulfate present in wastewater [63, 115]. This distinction was attributed to the varying metabolic processes that occur under these two distinct environmental conditions. Two principal mechanisms are responsible for this degradation. The first involves deconjugation, wherein bacteria present in wastewater synthesize arylsulfatases capable of cleaving the thioester bond at the C3 position of 17α-estradiol-3-sulfate, thereby releasing 17α-estradiol into the environment [67, 91]. The second mechanism is an oxidation process in which 17α-estradiol-3-sulfate is transformed into estrone-3-sulfate in the presence of oxygen [116, 117]. Aerobic degradation is predominantly characterized by oxidation at the C17 position of the 17β-estradiol-3-sulfate ring, rather than cleavage of the thioester bond at C3 [118]. Diverse degradation products, such as 6-keto-E1, 9,11-dehydro-E1, and estrolactone, have been identified in E2-3S,

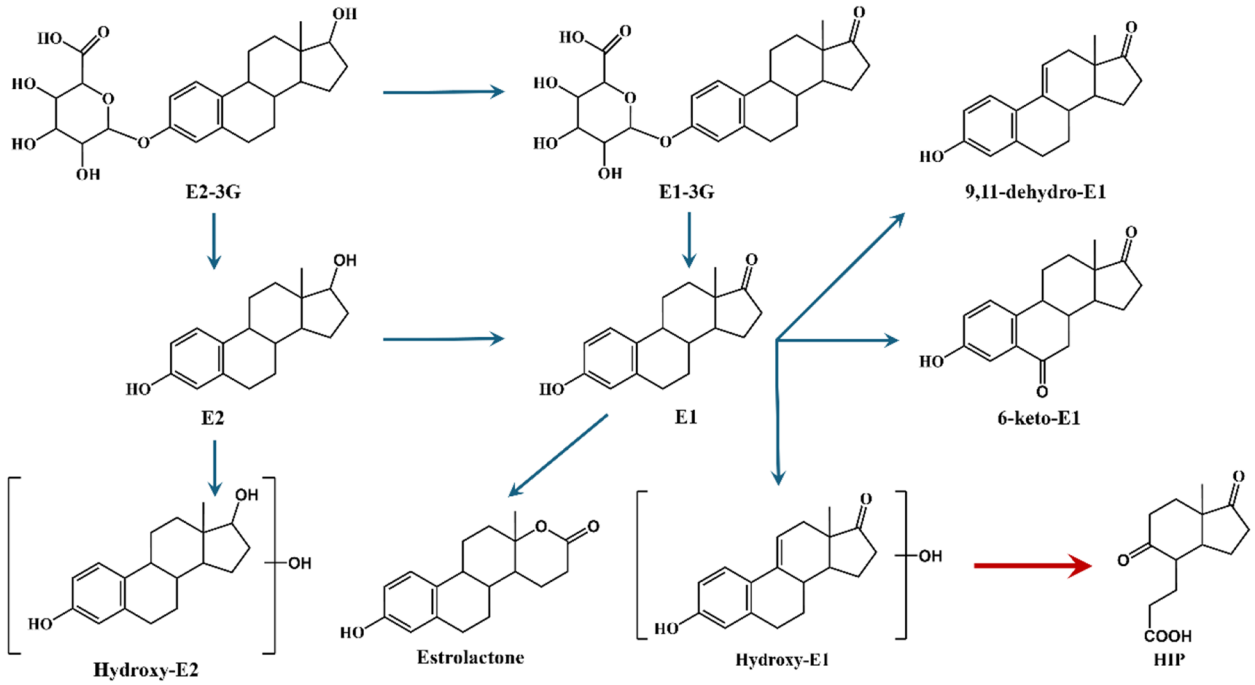



**Fig. 2** Pathways for biotransformation of E2-3G [84, 110–114]

highlighting a divergence from the degradation profile of E2-3G [118]. The degradation of E2-3S was particularly notable for the formation of various sulfate-conjugated intermediates (Fig. 3a). This includes the potential identification of sulfate-conjugated hydroxy-E1 and sulfate-conjugated keto-E1 as the degradation products. Under aerobic conditions, E2-3S undergoes initial oxidation to E1-3S [39]. Subsequently, E1-3S is deconjugated, and E2 is oxidized to form E1 [81, 119–121]. Nevertheless, the degradation of sulfate-conjugated estrogens is impeded by the constrained activity of arylsulfatases in the environment, resulting in a significant fraction of estrogens undergoing hydroxylation and ketogenesis [60, 82, 105]. Consequently, the biotransformation of sulfate-conjugated estrogens within wastewater treatment systems is intricate.

The anaerobic degradation of S-CEs proceeds at a slower pace than that of aerobic processes [82, 118, 122]. In anaerobic settings, deconjugation emerges as the predominant mechanism, with markedly diminished oxidative conversion. The initial product of the E2-3S degradation was E2, as depicted in Fig. 3b, with only a marginal generation of E1-3S [118]. Under oxygen-deprived conditions, both E1-3S and E2 are susceptible to thioester bond cleavage and subsequent oxidation, leading to the formation of E1 [62, 82, 123]. Consequently, E1, which appears as a secondary degradation product, originates from the metabolic conversion of E2 and E2-3S.

## Factors affecting the microbial degradation of ces

Biodegradation has been demonstrated to be an effective strategy for the elimination of CEs, a process that was intrinsically linked to the action of microorganisms. The influence of various environmental factors on the biodegradation of CEs has been delineated, with some factors directly impacting enzymatic activity and others shaping the composition of microbial communities. The summarized findings in Table 5. underscore the significant impact of these factors on the efficacy of CE removal.

### *Temperature*

The removal rate of CEs is significantly related to temperature, as temperature can influence the activity of arylsulfatase and β-glucuronidase as well as the abundance of bacteria containing these enzymes, which in turn affects the deconjugation process of CEs [39, 124]. A robust correlation has been identified between the increase in temperature and the concomitant decrease in the concentration of conjugated compounds within the aqueous phase of activated sludge, with a coefficient of determination ($R^2$) reaching an impressive 0.994 [62]. Upon elevating the operating temperature from 15 to

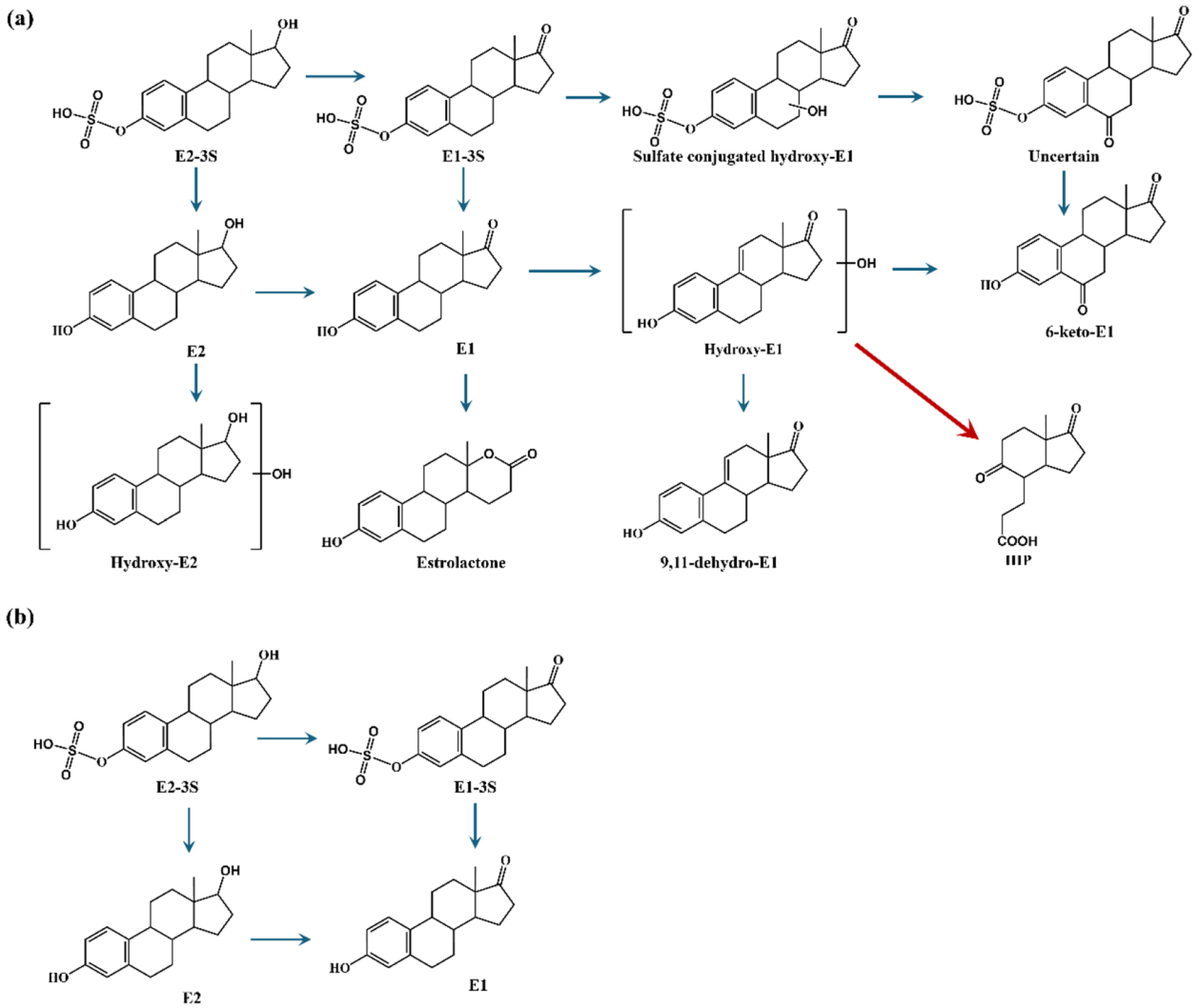



**Fig. 3** Pathways for the biotransformation of E2-3S under aerobic conditions (**a**) and anaerobic conditions (**b**) [82, 115–123]

**Table 5** Factors affecting the biodegradation of CEs

| Factor | Correlation | | Reference |
|---|---|---|---|
| | $R^2$ | *p* | |
| Temperature | 0.731 | 0.005 | [19] |
| pH | 0.73-0.99 | - | [66] |
| Mixed liquor suspended solids | 0.94-0.99 | - | [66] |
| Solids retention time | 0.36-0.594 | 0.005 | [19, 51] |

35 °C, the aerobic degradation rate of 17α-estradiol-3-sulfate increased by a factor of approximately 92. By contrast, the corresponding anaerobic process exhibited an 11-fold increase [118]. In two full-scale wastewater treatment facilities, a decrease in temperature of 6 °C led to a 20% decrease in the removal efficiency of E1-3S [125].

The degradation rate of estrogen conjugates at different temperatures can be represented by a pseudo-first-order kinetic model [67]:

$$\ln([C]) = -kt + \ln([C]_0) \tag{6}$$

where $k$ is the temperature-dependent biodegradation rate constant of 17α-estradiol-3-sulfate, $[C]$ is the concentration of estrogen, and $[C]_0$ is the initial concentration of estrogen. Within the temperature range of 15–35 °C, the influence of temperature on the degradation rate of estrogen conjugates can be quantified using the Arrhenius equation [117]:

$$\ln k = \frac{-E_a}{RT} + \ln A \tag{7}$$

where $A$ is the pre-exponential factor, Ea is the activation energy, $R$ is the universal gas constant, and $T$ is the absolute temperature (in Kelvin).

#### *DO and ph*

In addition to temperature, dissolved oxygen (DO) levels in the environment also significantly influence the biological removal of CEs [20, 118]. Arylsulfatases possess a highly conserved amino acid sequence that forms the active site after post-translational modification of cysteine or serine residues into $C_{\alpha}$-formylglycine residues, and it is only after this mechanism that arylsulfatases can exert their activity [126, 127]. However, this mechanism is an oxygen-dependent process that requires molecular oxygen as a cofactor; hence, the biodegradation of sulfate-conjugated estrogens in wastewater is an oxygen-dependent process [74]. Studies have shown that more than 95% of E2-3S is degraded under aerobic conditions, whereas under anaerobic conditions, the degradation rate is only 25% [118]. The majority of estrogens exhibit swift biodegradation under aerobic conditions, with half-lives typically not exceeding one day across a range of environmental substrates [128–130]. Conversely, the rate of anaerobic degradation of E2-3S was markedly lower than that of its aerobic counterpart. Consequently, the reduced degradation efficiency in anaerobic settings can potentially contribute to the prolonged presence of these estrogens in specific environmental contexts.

Environmental pH significantly influences the microbial degradation efficiency of CEs due to its impact on both the adsorption of substances by activated sludge and the activity of biological enzymes. The adsorption capacity of the sulfate functional groups present in E1-3S and E2-3S is inversely related to the environmental pH [66]. Specifically, at low pH values, sulfate ions form robust complexes with certain surface sites, which are not easily desorbed [131]. However, the presence of some organic ligands facilitates the removal of sulfate in acidic conditions more effectively than in neutral or alkaline environments [66]. Consequently, under acidic conditions, sulfate functional groups are likely to establish strong sulfate bonds with activated sludge, in addition to hydrogen bonding, thereby enhancing the adsorption of S-CEs [66]. Moreover, the enzymes implicated in deconjugating CEs, such as β-glucosidase and arylsulfatase, demonstrate pH-dependent activity, with optimal performance observed under acidic conditions(pH < 6.2) [132]. This pH dependency underscores the importance of environmental pH in the biological treatment processes for the degradation of CEs in wastewater.

#### *MLSS and SRT*

The mixed liquor-suspended solids (MLSS) have a significant impact on the biodegradation rate of CEs [133]. The Michaelis–Menten Model (Eq. (8)) can effectively describe the degradation kinetics of CEs at various MLSS. As the MLSS increases, the deconjugation rate of CEs becomes higher (Table 6) [48]. This may be due to the higher concentrations of extracellular and intracellular enzymes in the sludge system at higher MLSS levels, allowing bacteria in the activated sludge to utilize CEs.

$$\frac{dC}{dt} = -\frac{V_m \times C}{K_m + C} \tag{8}$$

where $C$ is the concentration of a chemical compound in a sludge solution, $V_m$ is the maximum reaction rate, and $K_m$ is a Michaelis constant.

Solids retention time (SRT) shows a significant correlation with the removal efficiency of CEs ($R^2 = 0.36–0.594$, $p < 0.05$) [19, 32], particularly for E1-3S and E2-3S, which seem to have the highest removal rates starting from the 8th to 9th day of SRT (> 99% removal efficiency) [32]. A longer SRT allows the sludge to develop a more diverse microbial community and enrich slowly growing microorganisms that may degrade CEs [134]. Additionally, the relative abundance of hydrophobic bacteria increases, which helps to enhance the adsorption capacity of the activated sludge for CEs [19]. Therefore, as the age of the sludge increases, that is, with the rise of SRT in WWTPs, the removal efficiency of CEs is improved [135].

## Conclusion and outlook

This article reviews the biodegradation of CEs in wastewater, providing an overview of CEs-degrading bacteria, transformation mechanisms, potential pathways, and influencing factors. Although some CEs-degrading strains have been isolated, our understanding of the biodegradation products and metabolic mechanisms of CEs is still relatively limited at present. Future research needs to reveal more CE biodegradation pathways and related genes to promote a deeper understanding of this field. Biodegradation has been proven to be

**Table 6** Deconjugation rates of CEs at different MLSS concentrations

| Compounds | Parameters | MLSS (g/L) | | |
|---|---|---|---|---|
| | | 4 | 7 | 12.5 |
| E2-3G | $V_m$ | 141.1 | 317.70 | 382.70 |
| | $K_m$ | 96.02 | 108.10 | 92.07 |
| E2-3S | $V_m$ | 26.12 | 36.60 | 57.02 |
| | $K_m$ | 50.40 | 61.17 | 62.36 |

the main pathway for the transformation of CEs and is expected to develop into a promising CE removal technology. However, research in this field still faces some challenges, which leads to most studies remaining at the laboratory stage. First, under complex environmental conditions, multiple microorganisms coexist, and current research on CEs in wastewater mainly focuses on detection methods and environmental behavior. The role of microbial species and their interactions in the biodegradation process of CEs needs further study. When necessary, integrated comics approaches can be used to deeply explore the biodegradation mechanisms of microbial communities. Another challenge for the biodegradation of CEs is the presence of various types of organic pollutants in wastewater. CEs often coexist with other pollutants in the environment, so developing microorganisms that can degrade multiple pollutants simultaneously has important research value. Finally, strains with degradation functions require further research and exploration, their viability, persistence, optimal operating conditions, impact on local microbial communities, and potential ecological risk assessments in practical applications. These factors are crucial for the transformation of CE biodegradation technology from the laboratory to practical applications.

**Abbreviations**

| | |
|---|---|
| SEs | Steroid estrogens |
| CEs | Conjugated estrogens |
| FEs | Free estrogen |
| G-CEs | Glucuronide-conjugated estrogens |
| S-CEs | Sulfate-conjugated estrogens |
| WWTPs | Wastewater treatment plants |
| MW | Molecular weight |
| E1 | Estrone |
| E1-3G | Estrone glucuronide |
| E1-3S | Estrone sulfate |
| E1-3S | Estrone sulfate |
| E2 | Estradiol |
| E2 | Estradiol |
| E2-17G | Estradiol glucuronide |
| E2-3G | Estradiol glucuronide |
| E2-3S | Estradiol sulfate |
| E3 | Estriol |
| E3-3G | Estriol glucuronide |
| E3-3S | Estriol sulfate |
| EE2 | Ethynylestradiol |
| EE2-3G | Ethinylestradiol glucuronide |
| EE2-3S | Ethynylestradiol sulfate |
| $LogK_{ow}$ | Logarithmic value of the octanol–water partition coefficient |
| DO | Dissolved oxygen |
| MLSS | Mixed liquor suspended solids |
| SRT | Solids retention time |

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**Authors' contribution**
WZ wrote main manuscript text. QMY prepared figures 1 and table 1-2. JJG provided topic, reviewed and edited manuscript. All authors reviewed the manuscript.

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All the data generated or analyzed during this study are included in this published article.
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## Declarations

**Ethics approval and consent to participate**
Not applicable.

**Consent for publication**
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The authors declare no competing interests.

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