TREATMENT AND REMOVAL STRATEGIES FOR ESTROGENS FROM WASTEWATER

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ABSTRACT

Natural and synthetic steroidal estrogens (estrone, 17β-estradiol and 17α-ethinylestradiol) are endocrine disrupters, that are discharged consistently from the sewage treatment works into surface waters, thereby causing endocrine disrupting effects to aquatic organisms at trace concentrations (nanogram per litre). Several years of research have been focused on their fate, behaviour and removal in the environment but primarily in the sewage treatment works which acts as a sink for these compounds. This review attempts to summarize the factors involved in the removal of these chemicals from the sewage treatment works. Biological processes, and to a limited extent physio-chemical properties, play a vital role in the endocrinal deactivation of which these compounds. The efficiency of these processes is highly dependent on operating parameters (such as sludge retention time, redox potential, etc) that govern the secondary treatment process of a functional sewage treatment works. Although advanced treatment technologies are available, cost and operational considerations do not make them a sustainable solution.

INTRODUCTION

Definition

Organic micropollutants in the aquatic environment have been extensively reviewed [1, 2, 3] however the fate and behaviour of endocrine disrupting chemicals (EDCs) have been less studied. Endocrine disruption (ED) has been evident since the 1930s [4, 5]. Many EDCs have now been reported as environmental contaminants [6, 7, 8, 9, 10, 11, 12, 13]. Natural and synthetic estrogens: estrone (E1), 17β-estradiol (E2) and 17α-ethinylestradiol (EE2) are such endocrine disrupters and they display the strongest estrogenic effects however their concentrations are lower in the aquatic environment [14], than phyto- and xenoestrogens [15]. The estrogenicity in the aquatic environment is largely due to their presence in the sewage treatment work (STW) effluent [16, 17, 18, 19, 20]. Many of these steroids pass through the wastewater treatment systems and are discharged continuously into the environment, mainly into surface waters. These compounds are amenable to biotransformation and bioconcentration [21] and potentially may bioaccumulate [22]; as a consequence of this behaviour complex issues for environmental health arise [23, 24]. Worldwide reuse of surface water for potable water production is increasing and a significant share of this is from wastewater effluent.

Although it is unlikely that natural estrogens will affect the human system, several studies have demonstrated endocrine effects such as feminization of male fish linked to these estrogenic compounds in STW effluents. Typical concentrations of unconjugated steroid estrogens found in STWs influent and effluents in different countries are presented in Table 1.

There are broadly two different approaches for removal of steroid estrogens and other organic micropollutants from the wastewater: optimizing existing treatment technology; and upgrading existing sewage treatment plants with new end-of-pipe technology. This review attempts to highlight some of these technical possibilities and current technologies that are available to tackle the issue of natural steroid estrogens in the environment. References have been confined to wastewater treatment works as most research focuses on the cause and effects on the aquatic life near or downstream from the wastewater effluent discharge. Figure 1 summaries the possible approaches to remove steroid hormones from source to the wastewater treatment works.

CONVENTIONAL WASTEWATER TREATMENT

Overview of STW

A conventional STW is typically a three stage process consisting of preliminary treatment, primary sedimentation and secondary treatment [25]. Wastewater treatment begins at the head of the works with preliminary treatment, typically inlet screens. However steroid biodegradation and biotransformation have been found to occur before the STW within the sewage system. This arises from the presence of bacterial slime which accumulates on the walls leading often to anaerobic biodegradation. In large catchment areas, the retention time of the sewage system can be significant allowing a high degree of transformation and degradation [26]. Conventional wastewater treatment provides the best model to study the mechanisms through which natural estrogens are attenuated in nature and engineered systems. It is generally believed that transformation and biodegradation are the two main processes for estrogen removal from wastewater, with some disputing that adsorption can play a significant role in estrogen removal. Mastrup et al. [27] estimated that the biodegradation process accounted for less than 10% of the removal of natural and synthetic estrogens, even though a considerable amount is adsorbed to the sludge and the majority of the compounds remain soluble in the effluent. In this section, the steroid hormone removal will be described in the conventional treatment plant.

Preliminary Treatment

Initial raw sewage screening occurs at the preliminary treatment at the head of the works where large floating objects, grit and dense inorganic solids are removed. A small amount of organic material is removed from the screens. Little or no removal of organic micropollutants and steroid hormones is observed at this stage [18].

Primary Sedimentation

In the primary sedimentation tanks, the estrogen removal mechanism is reported to be adsorption. The degree of micropollutant removal depends largely on the hydrophobicity of the hormones, the suspended solids content and their subsequent settling, the retention time and surface loading. Lipophilic compounds such as fats, oils and greases can adsorb a significant amount of hydrophobic compounds, including many endocrine disrupters, which are removed. However, estrogens are polar and hydrophilic in nature suggestive of low adsorption. The $log\ K_{ow}$ (concentration ratio at equilibrium of an organic compound partitioned between an organic liquid and water) of steroid estrogens E1, E2, E3 and estrone-3-sulfate (E1-3S) are 3.4, 3.1, 2.7 and 0.95 respectively. Note that although E1-3S is not an endocrine disrupter, the cleavage of the sulphate moiety yields estrone which is estrogenic and has been found to be a significant contributor to the overall estrogenicity in receiving waters. Estrogen removal at this stage of the sewage train is thus often found to be insignificant. No E1 removal was observed in a Norway STW and Canada (E2 and E1) where only primary treatment occurred [28, 29]. In fact there was a reported increase of E1 from this Norwegian STW where only chemical addition was used for phosphorus removal. Similarly, a report from the United Kingdom suggests that E1 and E2 are not removed significantly from the primary sedimentation but rather from the post-primary sedimentation stage i.e. biological treatment process [30].

Secondary Treatment Processes

It has been shown that secondary biological treatment is the key process behind the ability of some STWs to remove most, if not all, estrogenic activity [31]. Transformation and biodegradation are thought to play a significant role in the hormone removal since some of the microorganisms present in biological STWs posses the potential to utilize steroid estrogens amongst other micropollutants as carbon sources for metabolism. The most rapid and complete degradation of pollutants present in the STWs is brought about under aerobic conditions through catabolic pathways [32]. Correspondingly, anaerobic, nitrifying–denitrifying and methanogenic bacteria that carry out the detoxification of effluent contaminants have been discovered from various STWs [33].

The population dynamics of such microorganisms depend greatly on the types of process i.e. trickling filter, activated sludge process, membrane bioreactors and enhanced biological process. This section focuses on the impact of biological treatment design on EDC removal, since that is the key component of a conventional STW for estrogen removal. A recent study in England showed particularly dramatic benefits of adding a biological step. Simply adding a short secondary treatment stage of fine bubble aeration to a domestic STW that previously had only primary settlement produced a sudden and sustained reversal in feminization trends in downstream fish [34].

Removal of steroid hormones using trickling filters

Trickling filters are generally less effective than activated sludge systems in eliminating estrogenic activity. Less than one third of the total estrogenicity was removed in a trickling filter system [35]. In a study to map the distribution of natural estrogens from 18 Canadian municipal treatment plants, Servos et al. [36] found poor estrogens removals at plants that incorporated trickling filter (Table 2). Elsewhere, elevated levels of E1, E2 and EE2 were found in effluent from a trickling filter, over two sampling periods [18]. This supports the work of Turan [37] who found that estrogens, particularly synthetic compounds, are stable enough to withstand the sewage treatment process. Conversely, a more extensive treatment train with two stage trickling filter process and two stage post sedimentation was able to achieve removals of 58% for E1 and 82% for E2 which was comparable to an activated sludge process [30]. Unfortunately, no information on the operating parameters was given that may contribute to the relatively high removal of the treatment works.

It is surmised that high removal rates may be achieved at plants with comprehensive treatment technologies such as combined biological and chemical removal of organic matter, nitrogen and phosphorus but not via the trickling filter process. This was evident in an observation involving two U.S. STWs where one plant is a Bardenpho BNR activated sludge system with a solids retention time (SRT) of 10-13 days and the other is a trickling filter system. Both plants receive primarily domestic influent and operate at similar temperatures. The activated sludge system exhibited a superior micropollutant removal compared to the trickling filter system. The difference in the removal performance has been thought to arise from the difference in the hydraulic retention times (HRT). Typically, the HRT of most European activated sludge systems is between 4 and 14 hours [38] compared to a trickling filter which might have an HRT of less than one hour.

Removal of steroid hormones using activated sludge process

Conventional activated sludge process is commonly used to treat domestic and industrial wastewater mainly to remove organic compounds in STW influent. Batch studies have indicated that E1 and EE2 will not be completely removed in activated sludge in the present process [38]. Field data suggested that removal performance for E1 varied but removals were greater than 85% for E2, E3 and EE2 [38]. Baronti et al. [39] reported average E1, E2, E3 and EE2 removals of 61%, 86%, 95% and 85% respectively in six STWs near Rome. Ternes et al. [18] found low elimination efficiencies for E1 and EE2 (<10%) but approximately two-thirds of E2 was eliminated in the STWs. This was in agreement with Komori et al. [40] who observed a 45% reduction in E1 which was considerably less than the reduction found for E2 and E3. The persistence of EE2 under aerobic conditions and rapid degradation of E1 and E2 were found also in the laboratory experiments using STW sludge. In two pilot-scale municipal wastewater treatment plants, removal efficiencies for E1 and EE2 were 60% and 65% respectively with elimination of more than 94% for E2 entering the aeration tank [41].

In activated sludge systems, solid retention time seems to be an especially important factor in EDC removal. Several researchers have noted improved removal with increased SRT [18, 42, 43]. A retention time of at least 10 to 12.5 days has been suggested as the period required for the growth of organisms that decompose E2 and E1 [44].

Removal of steroid hormones using membrane bioreactors

In existing STW where it may not be possible to adequately increase the SRT because of expense or site constraints, membrane bioreactors (MBR) could offer advantages of more flexibility to operate at higher SRTs in a smaller footprint. MBR technology is often considered a promising development in wastewater treatment which integrates biological degradation of waste products with membrane filtration. These treatment systems are effective in removing organic and inorganic compounds as well as biological contaminants from wastewater [45]. Steroids removal rates of greater than 90% were achieved in membrane bioreactors with nitrification and denitrification (SRT of 12-15d) [46]. The sludge originating from a membrane bioreactor (sludge age 30d) showed significantly higher activity in batch experiments by a factor of 2-3 than in conventional activated sludge with a solids retention time of 11d [46]. Unfortunately, analytical limitation prevented the confirmation of increased removal activity in full-scale plants.

Biological degradation has been cited as the important factor in the removal of estrogens and other endocrine disrupters in membrane bioreactor [47, 48]. This is in agreement with Ivashechkin et al. [49] who operated conventional activated sludge and MBR pilot units in parallel, operating both for denitrification at two different SRTs (12 and 25 days), and applying the same influent wastewater and sludge loading rate to each system. The authors did not find an appreciable difference in removal of EE2 between the two systems and determined that estrogen removal was due primarily to biodegradation; removed estrogens were not sorbed onto sludge particles, nor were they retained in the membrane material or the membrane biofilm. Weber et al. [50] report that E2 turnover rates to E1 did not differ greatly between conventional and membrane activated sludge. Furthermore, no degradation was observed for the persistent EE2 in both sludges. While microfiltration membranes themselves will not provide an enhanced degree of estrogens removal, it has been suggested that estrogens adsorption to particulate matter that is retained by the membrane would reduce estrogens concentration in the effluent. Some researchers have found that microfiltration membranes are able to display some retention of smaller particles or colloidal material onto which estrogens may adsorb [48, 51]. Since pore sizing of membrane material is not uniform between manufacturers, it is possible that a difference in membrane material may explain some of the discrepancies in colloid retention. Differences in detection limits may also play a role. The improved removals exhibited by these systems have also been attributed to low sludge load amongst the previously mentioned factors i.e. high SRT and HRT.

Removal of steroid hormones using biological nutrient removal plants (BNR)

Of the biological treatment plants, BNR employed for nutrient removal such as nitrogen and phosphorous exhibits significant overall estrogen removals. To achieve biological phosphorus removal, an anaerobic zone between the activated sludge and influent wastewater before aerobic degradation is necessary. Biological nitrogen removal involves nitrifying and denitrifying reactions. Nitrification results in the conversion of nitrogen from a reduced form (ammonia) to an oxidized form (nitrate). It is not in itself a significant nitrogen removal mechanism. Some anoxic volume or time must also be included to provide biological denitrification to complete the objective of total nitrogen removal and the various process configurations are discussed in detail elsewhere [52].

An activated sludge system for nitrification and denitrification including sludge recirculation has been observed to eliminate appreciably natural and synthetic estrogens [42]. These authors found that to a large extent, the natural estrogens were degraded biologically in the denitrifying and aerated nitrifying tanks of the activated sludge system, whereas EE2 was only degraded in the nitrifying tank [42]. This is in agreement with Vader et al. [53] who demonstrated that the EE2 degradation capability of sludge correlated with the nitrifying activity. The complex redox reactions occurring within BNR systems was investigated by Joss et al. [46] who described the influence of redox conditions on the removal efficiencies of estrogens using a model. The maximum removal rate occurred under aerobic conditions when E1 was reduced to E2. An increase by a factor of between 3 and 5 was observed for the degradation of E1 under all redox conditions in the transition from anaerobic to anoxic as well as between anoxic and aerobic. The oxidation of E2 was observed also at a high rate under all redox conditions: the rate difference observed between anaerobic and aerobic systems was below a factor of 3. The reduction of E1 to E2 was shown to take place under anaerobic conditions without nitrate. The removal of EE2 occurs at a significant rate only under aerobic conditions. Estrogenic levels through the enhanced biological nutrient removal (EBNR) process train (SRT = 25.8 days) were detected using sheep estrogen receptor binding assay and the MCF-7 breast cancer cell proliferation assay [54]. The raw influent was highly estrogenic and arose from the presence of estrogens (>48%) as determined using chemical analysis. Estrogenicity increased slightly after primary treatment. Secondary activated sludge treatment including nitrification/denitrification removed >95% of the estrogenic activity. Subsequently, estrogenicity was found to be below the detection limits in the tertiary-treated effluent [54]. In two Swedish STWs that incorporate nitrogen removal with ASP, the removal of steroid estrogens (based on EEQ ng l^{-1}) from the effluent was >97% [35]. Removal of up to 19.5 ng l^{-1} was found and activated sludge, especially in combination with subsequent anoxic stages to achieve nitrification, was most effective due to the prolonged duration of the biological treatment. However, in a survey carried out on the distribution of E2 and E1 in 18 selected municipal effluents across Canada, no statistical correlation was found between the HRT or SRT and the apparent steroid removals even though plants or lagoons with high SRT exhibited high steroid hormones removal efficiencies. In addition, these workers found that nitrifying plants exhibit greater removals than those without nitrifying capabilities [36].The presence of aerobic, anoxic, anaerobic zones in the BNR allows for most of the removal processes i.e. anaerobic biodegradation, adsorption, anoxic biodegradation and aerobic biodegradation to occur. A high sludge age is usually required to achieve nitrification and nutrient removal because the autotrophic bacteria involved grow very slowly.

Integrated Fixed Film Activated Sludge System (IFAS) combines fixed-film and suspended activated sludge processes. The advantages for this configuration include less tankage requirement, lower capital cost, modular systems allow for incremental additions of modular components which may alleviate short- and medium-term financing requirements, little or no additional operation costs or operator attention compared with conventional activated sludge. Johnson and Darton [55] proposed to increase locally the amount of biomass sorbent by providing a carrier material within the activated sludge basin onto which a biofilm can develop. A wide range of mild to strongly hydrophobic organic contaminants would be intercepted by the bacterial surfaces and biodegraded. They propose a fixed surface rather than mobile carrier particles to ensure contact of influent wastewater with the biofilms. The fixed matrices would be located toward the front end of an aeration tank (some degree of plug flow is desired) and would be laid out in several packed zones. Laboratory scale tests have shown that almost all steroid estrogens can be removed by this process at a modest extra cost to existing facilities. Joss et al. [46] found removal rates of 77% and ≥90% for E1 and E2 in a fixed bed bioreactor of a low hydraulic retention time of 35 min. Since the HRT may have little impact on steroid estrogens removal capability, the authors suggested that the high age of the biofilm sludge was one of the main reasons for the observed removal.

INFLUENCE OF BIOLOGICAL FACTORS AND OPERATING PARAMETERS ON ESTROGEN REMOVAL

Importance of Microbiological Population

The nature of adapted microbiological populations is an important variable in the removal of estrogens. Layton et al. [56] reported that the removal efficiency of E2 in biosolids obtained from municipal plants was 80% greater than removal efficiency found in biosolids obtained from industrial plants.

Biotransformation batch test system using an activated sludge showed little or no EE2 transformation over 20h. However, in another laboratory study involving nitrifying activated sludge where the energy sources were ammonium and hydrazine, good removal of EE2 (circa 28h half-life) was obtained. The removal rates of EE2 in nitrifying activated sludge were at a maximum for the first 2 days with a degradation rate of 1 $\mu g\ g^{-1}$ Dry Weather h^{-1} [53]. After this period, the degradation rate slowed. This was likely due to the affinity of microorganisms for EE2 at lower concentrations or to a decrease in the activity of the nonproliferating cells. The low concentration may result in the bacteria being in a starved condition (senescent cells) in the phase between death and the breakdown of the osmotic regulatory system (in moribund state) [25]. This led Vader et al. [53] to surmise that successful elimination can occur with STW that favour nitrifying bacteria with long SRT and warm summer temperatures. This notion is supported by Layton et al. [56] who found that sludges that failed to nitrify significantly also failed to biodegrade EE2.

The activated sludge process of the biological plant has been reported to be responsible for the biotransformation of mestranol to EE2 [57]. Mestranol was degraded after a lag-phase of approximately 5 h and yielded a turnover rate of approximately 80% after 24 h. The only metabolite found was EE2, at a concentration of 7 % with regard to the initial concentration of mestranol. Unfortunately, no bacterial strains were identified to be responsible for the biotransformation. The biotransformation of mestranol to EE2 is in contrast with the results obtained by Norpoth et al. (1973) [58] who reported mestranol persistence over 120 h in their batch experiments.

In another study, it was suggested there was a weak link between percentage removal for E2, E1 and estrogenicity activity associated with nitrifying STWs. Using nitrifying activated sludge (NAS) in the presence of ammonia-oxidizing bacterium *Nitrosomonas europaea*, it was observed that NAS degraded 98% of E2 at 1 mg l^{-1} within 2 h [33]. The research group also found that E1 was generated when NAS degraded E2, whereas E1 was not generated when *N. europaea* degraded E2. This is suggestive that E2 degradation via E1 by NAS is caused by other heterotrophic bacteria that exist within NAS and not by a nitrifying bacterium such as *N. europaea*.

Heterotrophic bacteria that have been identified in the environment to degrade estrogens include *Rhodococcus erythropolis* and *Mycobacterium fortuitum* [59, 60]. Using strains of *Rhodococcus* isolated in activated sludge from wastewater treatment plants, Yoshimoto et al. [61] found rapid degradation of high concentration (100 mg l^{-1}) of natural and synthetic steroid estrogens (E1, E2, E3 and EE2). Recently, *Sphingobacterium sp.* JCR 5 isolated from the activated sludge of STW was reported to be able to degrade EE2. This microorganism grew on EE2 as sole source of carbon and energy and metabolized up to 87% of the substrate added (30 mg l^{-1}) within 10 days at 30°C [62]. A novel gram negative bacterium *Novosphingobium tardaugens* has also been isolated from activated sludge in Japan and found to have E2-degrading properties [63, 64]. The recently proposed genera *Novosphingobium* [65] was previously included in the genus *Sphingomonas* [66]. The genus *Novosphingobium* is well known for including many species that can assimilate biodegradation-resistant compounds. *Rhodococcus equi* and *R. zopfii* showed degradation of 100 mg of E2 per litre to 1/100 of the specific activity level after 24 h. This is in agreement with Yoshimoto et al. [61] who found rapid degradation of high natural and synthetic steroid estrogens (E1, E2, E3 and EE2) concentrations with *Rhodococcus* isolated in activated sludge from wastewater treatment plants. Two strains of microorgranisms, *Achromobacter xylosoxidans* and *Ralstonia picketii*, cultivated from membrane activated sludge on an enriched culture R1 were able to utilise E2 and E1 as growth substrates and transformed E3 but not EE2. Transformation rate of E2 by R1 was found to be 0.013-0.015 mg h^{-1} [50].

The number of organisms responsible for degradation, identified over 40 years from when *Streptomyces* sp. strains were initially reported as the microorganisms that perform the microbial transformation of E2 to E1, is increasing. Research into identification of strains of microorganisms that can break down estrogens with the possible application to wastewater treatment has led researchers to explore other areas. Shi et al. [67] have isolated from cowshed samples an EE2-degrading microorganism *Fusarium proliferatum* which uses EE2 as the sole source of carbon. They have reported the existence of EE2-degradative products, which may be more polar compounds that have a phenolic group, however these substances have yet to be identified.

Culturing these strains in bulk and introducing them into the biological reactors of wastewater treatment plants could potentially reduce the concentrations and estrogenic activities of estrogens released into the environment. By understanding the diversity of microorganism and enhancing their degrading activity, it can be envisaged that these steroids may be completely metabolized to carbon dioxide and water. However, there is little research in the literature on the known degradation pathway or metabolism of estradiol and estriol. A conjecture could be that the metabolism of the two natural steroid estrogens will have similar degradation pathways as E1 and probably EE2. Further study is required to identify intermediate metabolites and elucidate the complete mineralization of all these steroid hormones in the environment. In addition, although nitrifying plants have apparent elevated removal of the hormones and lower estrogenicity levels in the effluent, further tests are necessary to establish process and treatment characteristics contributing to the removal of these hormones.

The stability of the bacterial communities in activated sludge systems depends not only on operational parameters such as hydraulic retention time, and food to microorganism ratio (*F/M*) but also on the eukaryotic community.

Role of Precursors Conjugated Estrogens and the Respective Bacteria

Natural steroid hormones are often metabolized in the liver and excreted from the body in hydrophilic conjugates of the parent compounds. The natural and synthetic steroid estrogens are excreted in the urine of humans primarily as a variety of biologically inactive or non-estrogenic glucuronide or sulphate conjugates [56, 68, 69]. Research has shown that the majority of steroid estrogens enter the STW in their conjugated form. The primary source of unconjugated steroids to the aquatic environment is from the STW thus demonstrating that deconjugation occurs during the treatment [39, 70, 71]. .

Based on previous observations by several authors and the daily excretion of estrogens by humans factoring in the dilution factor, parts per trillion or ng l^{-1} can be expected to be present in the aqueous environmental samples [16, 71, 72]. Johnson et al. [72] used a simple prediction method and suggested that the amounts of E1 and E2 arriving in the sewage influent were close to those which may be excreted by an "ideal" population following deconjugation. However, the concentrations of hormones in the raw sewage entering the STW are highly variable. It was found in an extensive survey of influent produced by 3500 to 1.2 million population that the range of E2 was <0.5 to 48 ng l^{-1}, E1 was 17 to 102 ng l^{-1} and E3 from <0.5 to 10 ng l^{-1} [72]. Samples of raw sewage analysed from five STW for unconjugated steroids was 7 ng l^{-1} for EE2, 1.5 ng l^{-1} for E2 and 5.5 ng l^{-1} for E1. After cleavage of conjugates the medians for total steroids were 9.5 ng l^{-1} for EE2, 3 ng l^{-1} for E2 and 13 ng l^{-1} for E1. This demonstrated the importance of steroid conjugates which can account for up to 50% of the total concentration in the influent [73]. Concentration of conjugated estrogens was high in a study conducted at 20 STW [40]. In the analysis of conjugated estrogens in STW influent, Komori et al. [40] found that the conjugated estrogens exist at higher concentrations in both the influent and the secondary effluent than stated in other studies [16, 18] and conjugated estrogens still remained at high concentrations even in the secondary effluent.

Deconjugation could occur through microbial processes in STWs [56, 57, 68, 71, 74, 75] and in rivers [74]. Batch reactor experiments have found that glucuronides of E2 were rapidly cleaved in contact with diluted activated sludge thus releasing the free E2. After no more than 15 minutes, the E2-glucuronide conjugate was cleaved and both E2 and E1 were released and detected [57]. The result of the increase in E1 concentration in the effluent during the conventional activated sludge process therefore could be explained by the oxidation of E2 in the aeration tank and by the cleavage of the conjugates [76].

The fate of estrogen conjugates in the municipal aqueous environment was investigated with measurement of concentration levels of free and conjugated natural estrogens in female urine in a domestic tank collecting wastewater from a block with approximately 250 inhabitants, as well as in six STW influents and effluents [77]. Results from this study showed that estrogen sulfates contribute 21% of the total conjugated estrogens. This percentage increased to 55% in the septic tank and was less dominated by estrogen glucuronides due to their susceptibility to the action of the already adapted *E.coli* with their abundance of β-glucuronidase. Although the influent of the STWs was dominated by free estrogens and sulphated estrogen species, the STWs were able to remove conjugated glucuronide estrogens (84 – 87%) however to a lesser extent E1 (61%) and E1-3-sulfate (E1-3S) (64%). Certainly the small fraction of E1-3S will be converted to E1 once released into the aquatic environment. The more commonly excreted E1-3S steroid conjugate has been observed to be more persistent because the arylsufatase enzyme is less common in the STW [39]. The origin of E1 in the sewage influent can be attributed to the byproduct of biodegradation of E2 in the sewer or alternatively it is largely due to deconjugation of E1-3-sulfate in the sewer. The conversion of E2 to E1 has to be considered in the overall degradation pathway of E1 effluent since it was found that no more than 69% of E1 was removed by activated sludge treatment [39, 78]. The outlet concentration of E1 even outnumbered that of the inlet in 4 out of 30 events [39]. Batch experiments indicated that E2 was rapidly transformed to E1 as the intermediate product [41, 78] and no other stable major metabolites were observed in the process in both aerobic and anaerobic reactors with activated sludge [79] as well as within MBRs [50, 79]. Biodegradation appeared to initiate at the D ring of E2, leading to the formation of the major metabolite E1. Depending on different sewage treatment works, the possibility remains that perhaps most E1-3S pass through the treatment works and into the receiving waters.

The common presence of β-glucuronidase enzymes from bacteria, especially *Esherichia coli*, which synthesized a lot of glucuronidase, excreted from the human gut (most common bacterium strain excreted by faeces to domestic wastewaters) can cause deconjugation of glucuronide steroid estrogens [57, 80]. It was found that E2-3-glucuronide conjugate was very readily converted to the active hormone E2 both in a semicontinuous activated sludge system (SCAS) and in the dechlorinated tap water used to dose the system [81], which suggests it has low survivability in the sewage system. Nevertheless further deconjugation was still taking place when Nasu et al. [82] (2001) showed that E2 concentration was increasing, before falling during biological treatment from raw influent to primary effluent within 27 Japanese STWs. A study of seven different STW effluents documents concentrations of EE2 ranging between 0.2 and 7 ng l^{-1} in three of the seven STWs [83]. EE2 is usually excreted out of the body as inactive conjugates (glucuronides and sulfates). The presence of unconjugated EE2 in some STWs suggests that deconjugation of EE2 exists in the stream of the STW. The pertinent problem is that EE2 is more stable than conjugated compounds and may persist longer in the environment. Although the concentrations of EE2 are very low (sub-ppt), the extreme potency of this synthetic estrogen means that even these concentrations could cause adverse biological effects.

In summary, the laboratory data suggest that some EE2 and E1 should be found in the effluent and expected to persist. However less E2 would be expected to emerge from the sewage treatment works. Assessing the conjugates from extrapolation data from laboratory, it seems that not all the conjugates are transformed before entering the STW; instead a proportion may deconjugate toward the end of the biological treatment yielding free steroids into the effluent. Ternes et al. [18] observed that 3% of the glucuronide conjugates still persisted after 28h in their batch experiments.

Operating Parameters

Organic loading, SRT and HRT

Joss et al. [46] hypothesized that substrate contained in primary effluent, competitively inhibits the degradation of the natural estrogens E1 and E2, implying that sludge loading is a key parameter influencing the removal of estrogens from a STW. This was confirmed by a low degradation rate observed in the first compartments of the monitored reactors. This means that microorganisms would prefer to degrade other organic compounds above estrogens. When low sludge load, in terms of biochemical oxygen demand (BOD) occurs, the microorganisms are forced to mineralise poorly degradable organic compounds. As such, Joss et al. [46] expected a reactor cascade to give better E1 and E2 removal rates than a completely stirred tank. However, no clear correlation can be found within one STW between different organic loadings and the removal of estrogens. Onda et al. [78] could not establish a clear correlation between E1 removal and loadings, despite the tendency for E1 removal to be lower under higher loads in most cases. Johnson et al. [72] tried to find a correlation between the flow per head and the E2 removal. Using the data from Svenson et al. [35] to find a correlation between the percentages of the yearly mean flow during the time measured, a trend can be observed showing a decrease in total estrogen removal with increasing percentage of flow, also indicating higher loading. In the review on MBRs for wastewater treatment, the enhanced elimination efficiencies of MBRs with respect to EDCs have been attributed to the low sludge loading amongst other possible factors [84] for example longer SRT.

An increase in SRT may enhance the biodegradative and sorptive capacity of the activated sludge. A high SRT in a biological process allows for a more diverse and specialized microbial culture to grow including slow growing microorganisms adapted to remove EDCs. Several researchers have noted improved EDC removal with increased SRT [18, 42, 43]. The influence of increased SRT is illustrated by a German STW which has been upgraded from a BOD removal plant to a nutrient removing plant, with substantial higher sludge retention times, increasing from <4 days to 11– 13 days. Batch experiments with sludge from the old plant did not show any reduction of EE2 [57], while at the increased SRT a reduction of around 90% was established in the full scale plant, which can indicate the growth of micro- organisms capable of degrading EE2 [42]. Further evidence of the effects of SRT on natural estrogens elimination from the wastewater is reported by Clara et al. [85]. At $SRT_{10°C}$ higher than 10 days, almost complete estrogen (E1, E2 and E3) removals were achieved and concentrations found within the range of their instrument's detection limits. The critical $SRT_{10°C}$ reported was between 5 and 10 days. However, no critical $SRT_{10°C}$ could be established for EE2. Elsewhere, minimum SRT periods of 10 to 12.5 days have been suggested for the growth of organisms that decompose E2 and E1 [44].

The other influence of SRT within the biological process is to affect the physical nature of the flocs and their ability to act as sorbents. The hydrophilic or hydrophobic properties of the flocs, comprised of bacterial aggregates, depend largely on their make-up which in turn is dependent on the microbial population and its growth rates. The exo-polymer coatings, comprised largely of polysaccharide and proteins, around the flocs would have an important effect on their affinity as sorbents for such compounds as E2. The nutrient status of the flocs could also influence the hydrophilic-hydrophobic balance [86]. Layton et al. [56] showed that the radiolabelled ^{14}C E2 sorbed the least to biomass from a low SRT (3 days) facility. Holbrook et al. [43] also reported a similar correlation of high SRT to high estradiol activity (µg E2-Eq kg^{-1} TSS) of the wastewater because of increased sorption. Holbrook et al. [87] attempted to correlate sorption behaviour with colloidal protein and polysaccharide concentrations but were only marginally successful (r^2 similar to 0.4). These low correlations suggest that aromatic content, protein, or polysaccharide concentration can not adequately explain E2 and EE2 sorption behaviour to colloidal organic carbon (COC) and that other fractions of the organic matter pool play an important role in binding. A substantial portion of the aqueous E2 and EE2 concentrations, up to 60%, may be associated with colloidal material, suggesting that COC may play a role in the fate and transport of E2 and EE2 during the activated sludge process [87]. Sorption of E2, EE2 and E3 onto several organic colloids was examined by Yamamoto and Liljestrand [88]. The sorption coefficient (K_{oc}) for humic substances and tannic acid suggested approximately 15 to 50% of the steroid estrogens was bound in typical natural water of 5mg total organic carbon (TOC) l^{-1}. Although the natural organic matter (NOM) may be higher in the sewage effluent, it is unlikely that sorption onto colloidal materials will be a major removal mechanism for natural steroids removal. These authors estimated that only 0.2 ± 0.06%, 0.24 ± 0.10% and 0.29 ± 0.07% of the total concentration of E1, E2 and EE2 respectively sorbed to suspended sludge particles (suspended solids concentration of 5 mg l^{-1} dry solids) in the effluents from a typical Danish STW [89]. In the presence of excess sludge, the removal of steroid estrogens was estimated to be 1.5-1.8% of the total loading in equilibrium conditions. The authors conclude therefore that sorption is not important for the fate of natural estrogens in STWs compared to biodegradation.

However ambiguity exists on the removal processes mechanism of steroid hormones. Johnson et al. [72] could not determine whether biodegradation or sorption was the most important removal mechanism of these hormones although other authors have implicated that E2 is adsorbed whilst E1 is biodegraded. A mass balance study in a Germany STW demonstrated that most of the estrogenic activity in the wastewater was biodegraded during treatment rather than adsorbed onto suspended solids [42]. Quantitative measurement with a miniaturized E-screen assay in E2 equivalent concentrations (EEQ) revealed that there were 58 and 70 ng l^{-1} in the influent and 6 ng l^{-1} in the effluent, indicating that the load of estrogenic activity of the wastewater was reduced by about 90% in the sewage plant. Less than 3% of the estrogenic activity was found in the sludge [42]. Furhacker et al. [90] used radiolabelled E2 to study the estrogen fate in the STW. At low concentrations, the majority of the radiolabelled E2 remained in the liquid phase and did not adsorb to the solids.

Another factor, often cited by researchers, affecting the removal efficiencies of biological plants is the hydraulic residence time (HRT) [35, 38, 91, 92]. A long HRT allows more time for adsorption and biodegradation. Superior removal efficiencies of E1, E2 and EE2 were achieved at UK STWs with longer HRT of around 13 h compared to 2–5 h [91].

Concentrations of estrogens below the detection limit were obtained for a plant with an HRT of 20h and a plant, which included wetland, with an HRT of 7 days [35]. Plant operating at HRTs of between 2–8 h achieved 58–94% removal compared to 99% removal achieved in a plant with an HRT of 12 h. Cargouet et al. [92] found better removal for E1 (58%) and E2 (60%) in plants with an HRT of 10–14 h compared to a plant with an HRT of 2–3 h in which a removal of 44% for E1 and 49% of E2 was established. The existence of E2, E1 and EE2 in 17 different STWs across Europe was studied [28]. When E1 effluent values (as % of estimated influent concentration) were plotted against the different STW parameters on a double logarithmic scale, better E1 removal rates (i.e. lower percentage E1 remaining) appeared to be associated with longer total HRT and SRT (r^2 = 0.39, 0.28 respectively, $p<0.5\%$) and longer HRT in the biological part ($r^2=0.16$, $p<5\%$).

Despite the evidence presented, no strong statistical correlations could be established between HRT/SRT and hormone removal. Johnson et al. [28] observed a weak significant ($\alpha=5\%$) correlation between E1 removal and HRT or SRT. In a Canadian study [36], there was little or no statistical correlation ($r^2<0.53$) between HRT or SRT and hormone or estrogenicity removal for 9 conventional secondary plants and 3 tertiary plants. This was reported despite the observations that plants with high SRT (>35days) or HRT (>27h) had relatively high removal of E1 and E2 and reduction in estrogenicity while low SRT plants (2.7 and 4.7 days) had more variability and lower removal. These studies highlighted some evidence that increased HRT and SRT increases the removal of E1 and E2 and other endocrine disrupters alike within the STWs. It has also underlined the importance of biological activity associated with longer HRT and SRT.

Hence sludge age is an important parameter to consider when studying the removal of steroid estrogens from wastewater treatment processes. Since SRT is a primary design and operating parameter, engineers and operators have the ability to influence the sorption potential of the biological solids by adjusting the SRT.

Effect of temperature and rainfall on removal efficiency

Temperature and seasonal variation may affect the removal of estrogens from the sewage treatment works. Generally, a reduction in temperature leads to reduced STW treatment efficiency as the metabolic rate of microorganisms present in the various treatment trains slow down. During winter, higher effluent concentrations for both natural and synthetic estrogens have been observed [16, 71, 93, 94]. The reduced amount of the slow growing specified bacteria responsible for EE2 removal due to washout in winter-periods has also been cited as a factor. An illustration of the effects of temperature on the estrogen removals in a STW can be found in a recent study by Nakada et al. [94]. A comprehensive mass balance study was carried out on a Japanese STW [94] in summer and winter. Similar characteristics were found in both seasons: 1. little difference in the E1 flux in the influent between winter and summer survey periods; 2. the sum of the E1 fluxes in the effluent of the final sedimentation tank and in the return sludge line was significantly higher than that in the aeration tank. However, sulphated conjugated estrogens, E1-3S and E2-sulphate, were not degraded in the treatment processes and persisted in the return sludge to a greater extent in the summer (15 ng l^{-1} and 16.5 ng l^{-1}

respectively) than in the winter (2.1 ng l^{-1} and 4.4 ng l^{-1} respectively). Estrone was more effectively removed in the aeration tank in the summer compared to the winter. Short SRT (8.2d) was attributed to the ineffective removal of E1 from the STW. However, high efficiency in summer with an SRT of 6 d was effective in reducing E1. This can be attributed to the higher water temperature (27°C) thus allowing rapid growth of E1-oxidising microorganisms. Removal efficiency was high in removal of E2 in winter (70%) and summer (87%). In contrast, concentration of E1 increased by 740% during the cold winter period and 10% in summer. The authors also observed that the removal of total nitrogen was smaller in winter (26%) than in summer (60%), probably due to the effect of influent temperature on nitrification which could affect the diversity of microorganisms responsible for the degradation of these steroid estrogens [94]. Other works demonstrating the influence of temperature on the degradation of estrogens include the work carried out by Ternes et al. [18] and Baronti et al. [39]. Ternes et al. [18] attributed the poor E2 removal (64 %) found at a German ASP filter, compared to a Brazilian STW equipped with a parallel ASP and trickling which achieved 99.9% E2 removal, to the low temperatures during the German sampling period with 2°C on average compared to above 20°C in Brazil.

With the available data so far no correlation between the temperature and the E2 removal in full-scale treatment plants has been found [28, 72]. A regression analysis of the data for E2 removal versus temperature gave only a r^2 value of 0.031, indicating no correlation. These authors also showed that there was little evidence (correlation r^2 of 0.1) between E2 removal and flow per head for a STW [72]. Layton et al. [56] experimented with undiluted mixed sewage liquor and found only 20% EE2 mineralized compared to over 75% for E2 over 24h. In the municipal biosolids, 84% of E2 and 85% of E1 were mineralized in 24h compared to <4% in industrial biosolids. No correlation between BOD and suspended solids removal with mineralization was observed, although temperature was seen to have an effect. At different temperatures, no significant differences in first order rate constants were seen for EE2 but E2 was significantly different and even at cold temperatures, it was rapidly removed by biosolids. Little E2 could be observed in the laboratory experiment performed by Ternes et al. [57] where rapid transformation of 90% of E2 (initial concentration 1 µg l^{-1}) was converted to E1 within 30 minutes. Over the 4h test, E1 seemed to persist in the system but the researchers observed a 50% loss after 24h.

In one study, concentrations of natural estrogens were 7.5 fold higher in a drought year, compared with a non-drought year [95] and removal percentages ranged from 20 to 64% in the dry year and 88% in the wet year. This effect was also observed by Kirk et al. [91] when comparing the samples collected in August, when the amount of rain has been substantial, to samples collected in May and April. Apparently the higher influent concentration has a larger influence on the removal than has the increased HRT, which can be expected as less wastewater enters the STW. Another study suggests that greater rainfall led to dilution of effluent, resulting in a lower estrogenic response in fish [96]. These studies were short term or single sample investigations which did not report physiochemical characteristics of the effluent. Monitoring the changes in physiochemical characteristics of the effluent could determine the efficiency of the STW to increase degradation of estrogens thereby lowering the estrogenicity of the sewage effluent [20]. The authors [20] observed that variation in temperature and concentrations of nitrite and nitrate may have significant effects in the efficacy of removing estrogens from the effluent. Long term studies are necessary to discriminate between increased temperature and other contributory factors like microbial activity or rain events.

3.3.3 Fate of steroids in sewage sludge

Most of the environmental programs carried out to assess the presence and impact of natural and synthetic estrogens in the aquatic environment have focused on the environmental waters such as sewage effluent and river waters and to a lesser extent on sewage sludge. Although some estrogens are not removed in the biological sewage treatment and exist in the digested sewage sludge [97], only circa 5% of the estrogens exist in the digested sewage sludge [42]. Only a few studies have analysed estrogens in soils and sediments [97, 98, 99, 100, 101, 102]. .

REMOVAL OF STEROID ESTROGENS – END-OF-PIPE MODIFICATIONS

Many of the water treatment process technologies have been successfully transferred for use in the removal of steroid estrogens in the wastewater treatment process. Current treatment processes include coagulation, activated carbon, membrane separation and advance oxidation processes to name a few. Some of these technologies employed for the removal of steroid estrogens in the water environment are presented in this section.

Chemical Coagulants

Chemical coagulants such as ferric and aluminium salts have been employed to remove organic matter and have been used in studies to investigate their capability to remove estrogens Schäfer and Waite [103]. carried out a comparative investigation of common adsorbents used in the water and wastewater treatment industry and found that $FeCl_3$ and MIEX® are not very suitable for removing the majority of trace contaminants [103]. However high removal (>90%) can be achieved with powdered activated carbon when added in a sufficiently high dosage [103]. The EDCs removal is minimal during coagulation since the previous process tends to favour the removal of large and hydrophobic compounds. Indeed, the latter are generally responsible for subsequent adsorption and decantation processes of small-sized contaminants, such as EDCs. This is in agreement with Svenson et al. [35] who found poor removal efficiencies (ca 18%) for steroid hormones in the STWs with chemical precipitation using ferric or aluminium salts without biological treatment. Laboratory jar tests over a range of ferric chloride dosages and pH conditions showed that coagulation was ineffective at removing E1 from secondary effluent and only the combination of powdered activated carbon (PAC) and microfiltration could be effective for removal of trace estrone from water [104].

In addition, the use of coagulants, such as aluminium and ferric salts, is often deemed impractical due to the high costs and often is environmentally and economically unsustainable.

Chlorination

Chlorination has been used extensively in the United States as a disinfection and oxidation agent of reduced inorganic species such as Fe(II), Mn(II) and S(II) in the water and wastewater treatment processes. However, disinfection by-products (DBP) are often generated and some of these are known to have mutagenic and carcinogenic properties [105, 106, 107]. Some suggestions that DBP can be estrogenic have arisen also. Itoh et al. [108] indicated that chlorination performed at many STWs increases the estrogenic effect of NOM but also reduces individual highly estrogenic compounds. As such, these authors stressed that the overall estrogenic effect has to be evaluated as a sum of increased and decreased activity by chlorination. Recent studies showed that estrogenic activity is usually reduced as a result of chlorination. This is in agreement with several workers who observed the rapid decomposition of E1, E2, E3 and EE2 [109]. This was accompanied with the formation of some carcinogenicity and/or mutagenicity and/or estrogenic DBP namely 4-chloro-E2 and 4-chloro-EE2. Therefore it is important to identify the metabolites or products from the reaction of estrogens with available chlorine and their associated estrogenic activities [110, 111].

Due to EU policy and regulations and the conservation of aquatic wildlife in the river, chlorination is only applied in drinking water supply as a disinfection agent and not for the discharge of wastewater effluent. It has been suggested by Snyder et al. [112] that efforts to control EDCs by chlorination may be counterproductive since additional DBP, often suspected to have toxic properties and be present in much higher concentrations in STW effluent than EDCs, may be produced.

Membranes

Membranes can remove reliably most trace micro-pollutants depending on compound size, the chemistry conditions of the feed solution and membrane material. Several investigative studies on estrogen removal using membranes have found that size exclusion is important. Tight and small pore sized membranes (reverse osmosis (RO) and nanofiltration (NF)) can achieve up to 90 % removal whilst the big pore sized membranes (microfiltration and ultrafiltration) did not perform well [113, 114]. Results from a study to investigate the removal of 52 steroid estrogens using NF and ultrafiltration (UF) membranes revealed that many steroid estrogens are retained on the NF membranes due to both hydrophobic adsorption and size exclusion whilst the UF membrane typically retained only hydrophobic steroid hormones due mainly to hydrophobic adsorption [115]. .

The significant role adsorption plays in estrogen removal using membranes has been highlighted by several researchers [116]. Chang et al. [116] found significant concentrations of accumulated estrone on hydrophobic hollow fibre microfiltration membranes. However these workers were concerned with the lowered retention as the amount of estrone accumulates on the membrane surface leading to a potential breakthrough.

The presence of NOM and/or suspended particulates in solution can enhance retention due to the interaction with these estrogenic hormones. The nonpolar and hydrophobic nature of estrogens according to their log K_{ow} values indicates that these compounds will favourably absorb onto sediment and sludge. Since most organic micropollutants or steroid estrogens are small in molecular size, usually in the range from 150 to 500 Daltons, only those compounds that associate with particles or colloidal organic matter will be physically removed during MF and UF.

Although using membranes seems promising, there are many factors to consider. RO and NF systems are very expensive and produce a concentrated reject stream that requires further treatment. In addition, the membranes are susceptible to fouling which makes the process less efficient and the need for regular cleaning arises. Several studies are undertaken to alleviate fouling [117]. This still does not solve the problems of removing these EDCs as it seems that these processes are just transferring EDCs from one source to another i.e. from aqueous to solid phase. Adams et al. [118] pointed out that RO and NF systems are usually not an economical option at WTPs which would imply that they are not expected to be economical at STWs either.

Granular Activated Carbon

Granular activated carbon (GAC) is widely used for water and wastewater treatment and its ability to remove estrogen to varying levels has been demonstrated [119]. Adsorption depends on the properties of both the sorbent and the contaminant. The dominant mechanism for the removal of organic micropollutants, using the activated carbon adsorption system, is hydrophobic interaction. However, ion exchange interactions can occur also in the removal of polar solutes [120]. Copresent substances such as natural organic matter (NOM) in natural water or in STW secondary effluent can interfere and compete for adsorption sites thus decreasing the activated carbon (AC) capacity for micropollutants [104, 121, 122, 123]. Fukuhara et al. [124] found that the amount of E2 adsorbed was reduced to about one-thousandth in river water and municipal STW secondary effluent by the large amount of copresent substances that compete with E2 for adsorption.

In addition, the influence of site competition and pore blockages can reduce the adsorption capacity of target compounds by copresent NOM such as humic and fulvic acids [125]. Lower adsorption constants were found when equilibrium studies involving E1 and E2 were carried out using wastewater as compared to the values obtained with distilled water. Other factors influencing the performance of AC also include the presence of domestic and industrial surfactants that may exert a negative effect on the adsorption capacity of AC for estrogens. Zhang and Zhou observed that an increased concentration of surfactants causes a rapid decreased in E1 adsorption constant. The properties of surfactants make them a good solvent of sparingly soluble organic compounds and therefore may have an effect on the partitioning of such compounds [125].

Although high estrogen removal levels can be achieved with AC, the removal of the subsequent pollutant which was merely transferred from the liquid phase onto the activated carbon has to be taken into consideration. The adsorbent must be either disposed or regenerated. Further treatment would have to be considered prior to disposal whilst regeneration can be very costly and energy intensive.

Ozonation

Ozone (O_3) has been used effectively as a disinfectant and an oxidant. Ozonation can lead to the transformation of steroid hormones via two strong oxidants: molecular O_3 and hydroxyl radicals (HO·) [126]. The hydroxyl radical reacts less selectively with organic micropollutants whilst the more selective ozone reacts with amines, phenols and double bonds in the aliphatic compounds [127]. Due to the selective nature of ozone, it may require coupling with other advanced oxidation processes (AOP) such as UV/H_2O_2 [128], O_3/H_2O_2 [129] or O_3/UV [130]. Experiments using ozonation and AOP for the removal of various endocrine disrupters has been carried out for the past decade. By employing O_3, estrone and estradiol removals of 62 – 98% and 57 – 100% respectively were achieved in sewage treatment plant effluent [131]. After ozonation, Nakagawa et al. [132] found 0.2 ng l^{-1} and non-detectable concentrations of estrone and estradiol respectively from the initial influent concentrations of 9.7 – 28 ng l^{-1} and 3.0 – 21 ng l^{-1}. Huber et al. [133] reported a rapid 17α-ethinylestradiol removal when exposed to ozone with a second-order rate constant of 3×10^{6} M^{-1} s^{-1}. For most steroid estrogen compounds (E1, E2 and EE2), an O_3 exposure of only circa 2×10^{-3} mg min l^{-1} could achieve ≥ 95% removal efficiency [134].

The removal of estrogens is dependent on their initial concentrations, co-existing compounds and their reactivities toward ozone and OH radicals [135]. OH radicals are more prone to be consumed by co-existing compounds, whose concentrations are relatively high in environmental water. Ozone was not particularly effective at oxidizing iodinated contrast media compounds, and AOP combinations with ozone did not significantly enhance removal rates [136].

In all cases, the ozonation products or metabolites formed are currently unknown and are an important consideration to take into account especially when NOMs are often present in wastewater effluent. Bromate and brominated organic compounds are of particular concern when waters being treated contain bromide.

Manganese Oxide

Removal of 81.7% of EE2 was achieved by using manganese oxide (MnO_2) [137]. An initial concentration of 15 µg l^{-1} of EE2 was spiked into tap water and filtered through upstream bioreactors filled with MnO_2 granules. Rudder et al. concluded that high amounts of EE2 were removed due to the adsorption capacity of MnO_2 and its catalytic properties. Since the MnO_2 reactor was not yet saturated after 40 days of treatment, they concluded that EE2 had also degraded into other compounds [137]. This treatment shows promising results in removal of similar estrogen compounds due to the self-regenerating cycle of MnO_2. Therefore, this advanced treatment technique will be cost effective in the long run. Unfortunately the authors did not identify the EE2 metabolites or their estrogenic activity.

Ferrate

Ferrate (Fe(VI)) is often studied as an alterative oxidant in wastewater treatment as it can be used as a dual process of oxidising and coagulating . At the acidic conditions, the redox potential of ferrate(VI) ions is greater than ozone [138] and is capable of oxidizing phenol, amines and alcohols. The potential of using Fe(VI) to remove phenolic EDCs (EE2 and E2) in water treatment was investigated [139]. The determined second-order rate constants of EE2 and E2 oxidation by Fe (VI) at near neutral pH ranged from 400 to 900 M^{-1} s^{-1} which suggests that significant removal of phenolic EDCs can be acheived. However, when real wastewater was employed, a higher dose of Fe (VI) was necessary as competition for these ions occurred between the organics and EDCs within the wastewater matrix. This was observed for Kloten wastewater where a dose of more than 1 mg l^{-1} was needed to achieve 99% removal. Higher dissolved organic matter in the wastewater (DOC 5.3 mg l^{-1} as C) resulted in a higher Fe (VI) demand whereas Lake Zurich water (DOC 1.6 mg l^{-1} as C) only needed 0.5 mg l^{-1} to achieve 99% removal of EE2 and E2. Nevertheless there is potential for this chemical at low doses (a few milligrams per litre) to achieve significant transformation of EE2 and E2 in both natural water and wastewater containing high concentrations of natural organic matter [139].

UV irradiation

UV lamps have been used widely for microbial disinfection of water and wastewater. Several endocrine disrupters have been reported to be amenable to transformation during UV treatment as they have chromophores that encourage the adsorption of light at UV wavelengths [112]. Liu and Liu [140] examined the direct photolysis of two estrogens E1 and E1 in aqueous solutions under irradiation with UV disinfection lamp and high-pressure mercury lamp (UV-vis-light). Photolysis of both estrogens causes the breakage and oxidation of the (A) benzene ring to produce compounds containing carbonyl groups [140]. This is consistent with the results from Mizuguchi et al. [141] who found the gradual degradation of estrone and E2, in UV irradiation, to almost complete removal within 6h. Degradation of E1-3S, however, did not start within 2.5 hr but was completed within 6hr. This suggests that the sulfonic acid moiety at phenolic hydroxyl group play an important role in degradation [141]. Ohko et al. [142]used titanium dioxide (TiO_2) photocatalysis to abate or remove the estrogenic activity of natural and synthetic estrogens in wastewater. No biological intermediary products were produced and these authors concluded that the phenol moiety of the E2 molecule, which was the essential functional group to interact with the estrogen receptor, should be the site of the photocatalytic oxidation of E2 [142]. Estrogenic activity ought to lose its potency almost concurrently with the initiation of the photocatalytic degradation and the estrogenic activities of the intermediate products were negligible [142]. Photocatalysis and photolysis degradation of estrogens (E2, E3 and EE2) in aqueous matrix were also investigated although the former is more effective than UV light alone [143]. Addition of silver and platinum metals to an immobilised TiO_2 photocatalytic system showed no degradation or mineralisation of EE2 in water [144].

Irmak et al. demonstrated that UV coupled with O_3 is more effective than using O_3 only [130]. The combination of UV and O_3 decreased both O_3 consumption and transformation time. Similarly, EE2 and E2 were more effectively degraded utilizing UV/H_2O_2 advanced oxidation as compared to direct UV photolysis treatment [128].

The degradation of steroid estrogens using ^{60}Co γ-ray ionizing irradiation in a Japanese wastewater plant was investigated [145]. These workers found that 200 Gy (J kg^{-1}) were required for the elimination of estrogen activity of wastewater to below 1 ng l^{-1}. However, as with ozonation, the elimination dose of the estrogen activity depended on the presence of total organic carbons which hindered the exposure of steroid hormones to the electron beam.

DISCUSSION

Front End Strategies

Source separation is a relatively new concept that is potentially useful for concentrating micropollutants before they arrive at the STW. There remain many challenges and questions with the implementation of source separation of urine for the removal of steroid estrogens. One of the most important aspects to take into consideration is the removal of estrogens from the concentrated stream before discharging. Advanced process treatments such as activated carbon, AOPs and membranes have been suggested to remove these hormones. However the presence of high levels of organic content interferes with the adsorption process [146]. This problem would also mean that higher doses of oxidants would be necessary in the AOP, indirectly increasing the cost. As for the use of membranes, the physical separation only serves to further concentrate the estrogens and not destroy or transform them to harmless products. Source control may seem to be relevant to compounds that affect biological balance within the biological works such as antibiotics rather than those that do not, such as estrogens, hence this process may be unfeasible.

Biological Treatment Processes

E1, E2 and EE2 are all “inherently biodegradable” [55]. This would seem to suggest that given the optimum and ideal conditions, biological process treatment should suffice. From the evidence from the vast research, a more complete biodegradation would require a drastic increase in the SRT or HRT of existing STWs or a change in the configuration of existing STWs by introducing anaerobic and anoxic zones. This would involve upgrading the existing activated sludge basins to gain a significant amount of SRT. Under the circumstances where both cost and space are prohibitive, the application of advanced tertiary treatment technologies for many communities would be economically unfeasible; MBR and IFAS might pose the possible options. These processes maximise use of the existing facilities and have small footprints. Further they can achieve high SRTs in small tank volumes.

Most MBR installations are less than 10 years old; therefore, the design criteria for removing micropollutants using this technology are still evolving. Several researches have been carried out to revolutionize the configurations and design approaches of conventional MBR to adapt to BNR [147]. Annaka et al. [147] reported a new MBR process that conducts efficient simultaneous biological nitrogen and phosphorus removal and compared to conventional MBR processes, an approximately 27% reduction in operational and maintenance costs was achieved. This is also demonstrated in IFAS which can be configured to act as a BNR thus allowing the breakdown of more persistent EE2 that is favored under anaerobic conditions. Based on an Ontario STW report in 1996, upgrading a conventional plant is estimated to be around \$3,500,000 whilst using IFAS the cost estimates are in the range of $1,000,000 to $2,300,000 depending on the type of media used [148]. However, these interesting and innovative processes have not been tested out on the removal efficiencies of estrogens.

End of Pipe Treatment

Biological processes are usually the most cost effective means of removing organics from wastewater, but when these organics are toxic or non-biodegradable, physical and/or chemical methods must be used. These methods include adsorption, chemical oxidation, and membrane processes that have been used more typically for water treatment. Methods such as reverse osmosis and active carbon adsorption only transfer the pollution and do not solve the environmental problem. Chemical oxidation i.e. AOP, on the other hand, has been seen to be an effective alternative for removing low levels of estrogens. However, only a few AOPs are applicable in wastewater effluent and the application of these techniques for the treatment of large-scale effluents is not economically acceptable. The Fenton process, for example, is one process that showed a good removal of estrogens but is not deemed to be practical since such removals only occur at acidic conditions (pH 3) [149]. In that project, ozonation was deemed to reduce estrogens at feasible cost. Ozonation was compared with conventional activated sludge treatment which is a cheap but energy consuming process: a total requirement of <0.04€ and <0.3kWh per m^3 wastewater was estimated (for activated sludge wastewater treatment typically 0.5€ and 0.3kWh are required). These led to the conclusion that ozonation can reduce estrogens in treated effluent at a feasible cost. However, it should be noted that ozonation was carried out on treated effluent from a BNR plant and hence is for the purpose of a polishing step. This concurs with the notion that AOPs in application for the treatment of large-scale effluents are not economically acceptable and that a significant decrease of treatment cost could be obtained by the combination of AOPs with a biological process. Hence the compatibility of these two processes should be proved. The costs of some of these multiple-barrier treatment processes compared with biological processes have been tabulated by Scruggs et al. [136] based on Black & Veatch design experience. Table 3 shows a range of the equipment costs and operation and maintenance costs for each of the options.

The main use of AOPs or other end-of-pipe treatments is as a polishing step because the performance of these processes depends greatly on the quality of the water matrix i.e. organic content. Estrogen removal in studies using a wastewater matrix tends to be inferior to that achieved in studies using a clean water matrix. Higher doses of chemicals are often necessary in wastewater to achieve the same removal efficiencies that are normally found in clean water. This leads to higher energy requirements. Irradiation beam is novel and innovative but the installation of an electron beam accelerator and the high energy consumption of this instrument may not justify its use in the immediate future. As with irradiation beam, the use of UV is energy intensive. The typical doses required for the treatment of steroid estrogens may be several orders of magnitude higher than for disinfection (<5 to 30 mJ cm^2). Therefore the use of UV treatment of steroid estrogens may not be economically competitive compared to other advanced treatment methods. The possibility of a self-regenerating cycle of MnO_2 can makes this treatment cost-effective, because the matrix does not have to be replaced. No clogging of the column was observed but this aspect needs to be substantiated by experiments over longer time periods. In the same context, further work is needed to characterize the putative biogenically formed MnO_2 which can have considerably altered surface and sorption characteristics [150].

Another consideration to take into account is the possible disinfection by-products arising from chemical processes. Disinfection by-products arising from oxidation processes are often under-reported and they might pose problems which may deem the treatment process counterproductive. Of the AOP options, UV/peroxide, result in the lowest disinfection by-products formation, has been suggested [136].

Undoubtedly, advanced treatment technologies will remove these compounds and ameliorate the quality of effluent but they will inevitably result in large financial costs, and increased energy consumption and carbon dioxide emissions [151]. It will be environmentally sustainable therefore to consider alternative techniques; optimizing STW operating parameters such as increased SRT and HRT in conjunction with nutrient removal stages and varying redox conditions.

CONCLUSIONS

- Steroid estrogens are excreted from the body in large amounts and can reach the aquatic environment at concentrations up to sub nanograms per litre levels therefore their constant discharge and persistence demands that attention is focused on their removal or endocrinal deactivation in the STW.
- Biological processes within the STWs play a central role in removing most if not all estrogenic activity. Biotransformation, biodegradation and adsorption are the mechanisms responsible for estrogen removal.
- The efficiency of these processes is highly dependent on parameters such as HRT, sludge age, organic loading, redox potential and the cultivation of the right environmental microorganisms.
- Plant configurations characterised by such parameters i.e. enhanced biological processes (BNRs and MBRs) perform better at removing estrogens.
- Precursor conjugated steroid estrogens and transformation into metabolites are just as important in the removal equations or mass balances as analysis of the unconjugated steroid hormones in accounting for the total burden in the STW.
- Nitrifying bacteria and other heterotrophic microorganisms work in synergy to breakdown steroid estrogens.
- Physical and chemical treatment processes, to serve as polishing steps preceding biological treatment, can yield an additional decrease in hormones in the sewage effluent to ultra low levels however their costs and practicability require further optimisation.
- No studies have suggested that inorganic compounds such as the priority hazardous substances of the Water Framework Directive (heavy metals) may reduce the population of microorganisms that are responsible for metabolising these chemicals.
- Unless regulatory levels of estrogens are lowered, the process treatments

previously used in drinking water treatment, to destroy the disease-causing organisms, may not be necessary in the wastewater effluent.

- Efforts should thus be focused on determining the reasons behind the STWs success and optimizating the activated sludge process as a cost effective treatment process that does not generate additional side streams requiring further treatment and disposal.

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616e0000000000000000000000000000000040000002d0101000500000009020000000020d000000320a5a00bc0001000400bc0000
00a8144e0a20ce2d00040000002d0100000300000000 Figure 1. Approaches to removal of steroid estrogens from source to the end of the sewage treatment train.

Table 1. Steroid estrogen concentrations in STW influent and effluent

Sampling location	Influent concentration (ng l^{-1})				Effluent concentration (ng l^{-1})				Method for analysis	Ref
	E1	E2	E3	EE2	E1	E2	E3	EE2		
United	1.8 –	<0.3	-	<LOD[a]	<LOD[a]	<LOD[a]	<LOD[a]	<LOD[a]	SPE/GC-MS-MS	[152]

Kingdom	4.1								
United Kingdom	-	-	-	-	5.4-29	1.6-7.4	-	-	SPE/GC-NCI-MS	[153]
United Kingdom	-	-	-	-	15-220	7-88	-	1.7-3.4	SPE/GC-MS	[20]
United Kingdom[1]	-	-	-	-	1.4-76	2.7-48	-	n.d[b]-7	SPE/GC-MS	[71]
United Kingdom[2]	57-59	132-224	-	-	21-39	31-32	-	-	SPE/GC-MS	[30]
United Kingdom[3]	77.8-81	182.6-188.7	-	-	19.5-48	12.8-14.6	-	-	SPE/GC-MS	[30]
Belgium	-	-	-	-	<0.2	<0.6	-	-	SPE/GC-MS-MS	[28]
France	9.6-17.6	11.1-17.4	11.4-15.2	4.9-7.1	6.2-7.2	4.5-8.6	5.0-7.3	2.7-4.5	SPE/GC-MS	[92]
France	-	-	-	-	0.5-4.5	<1-5.7	-	-	SPE/GC-MS-MS	[28]
Finland	-	-	-	-	<0.3-2.8	<0.8	-	-	SPE/GC-MS-MS	[28]
Netherlands	11-140	9-48	n.d[c]	0.5-8.8	0.4-47	n.d[c] -12	n.d[c]	0.5-1.8	SPE/LC-ESI-MS-MS	[72]
Netherlands	20-130	17-150	-	<0.3-5.9	<0.3-11	<0.8	-	<0.3-2.6	SPE/GC-MS-MS	[154]
Netherlands	-	-	-	-	2.9-3.8	<0.8-1.1	-	-	SPE/GC-MS-MS	[28]
Norway	-	-	-	-	3.0-35	3.0-13	-	-	SPE/GC-MS-MS	[28]
Italy	0.5-75	0.5-20	2-120	0.5-10	n.d[d] -54	n.d[d]-7	n.d[d] -28	n.d[d] -2.2	SPE/LC-ESI-MS-MS	[72]
Italy	25-132	4.0-25	24-188	0.4-13	3.3-82.1	0.35-3.5	0.44-18	n.d[e]-1.7	SPE/LC-ESI-MS-Ms	[39]
Italy	44	11	72	-	17	1.6	2.3	-	SPE/LC-ESI-MS-MS	[77]
Italy	15-60	10-31	23-48	<LOD[f]	5-30	3-8	<LOD[g]-1	<LOD[h]	SPE/LC-ESI-MS-MS	[155]
Spain	<2.5-11.5	<5-30.4	<0.25-70.7	<5	<2.5-8.1	<5-14.5	<0.25-21.5	<5	SPE/LC-MS	[99]

Spain	2.4	3	-	<LOQ[i]	4.4	<LOD[i]	-	<LOQ[i]	SPE/GC-MS-MS	[76]
Denmark[4]	-	-	-	-	0.4-47	0.6-12	-	0.3-7.5	SPE/GC-MS-MS	[16]
Denmark[5]	-	-	-	-	0.1-11	0.4-1.8	-	0.2-2.6	SPE/GC-MS-MS	[16]
Brazil	40	21	-	6	-	-	-	-	SPE/GC-MS-MS	[18]
Canada	-	-	-	-	3	6	-	9	SPE/GC-MS-MS	[18]
Canada	19-78	2.4-26	-	-	1-96	0.2-14.7	-	-	SPE/GC-MS	[36]
Sweden	-	-	-	-	5.8	1.1	-	4.5	SPE/GC-MS	[17]
Sweden	-	-	-	-	<0.3	<0.9	-	-	SPE/GC-MS-MS	[28]
Japan	-	-	-	-	2.5-34	0.3-2.5	-	-	SPE/LC-ESI-MS-MS	[156]
Japan	-	5	-	-	-	<LOD[j]	-	-	SPE/ELISA	[157]
Japan	31.9-197	13.3-25.8	83-255	-	2.8-79.7	0.49-16.7	0.31-0.84	-	SPE/GC-MS	[158]
Japan	15.1-18.2	3.9-23.4	-	<LOD[k]	22.2-154	<LOD[l]-7	5.5-5.6	<LOD[k]	SPE/LC-MS-MS	[94]
Japan	30.9-70.4	18.9-30.9	174.0-730.9	-	1.2-21	n.d-0.5	2.1-23.5	-	SPE/LC-ESI-MS-MS	[78]
Austria	29-670	14-125	23-660	3-70	<LOD[m]-72	<LOD[n]-30	<LOD[m] -275	<LOD[m] -5	SPE/LC-MS-MS	[159]
Switzerland	7.3-75	4.9-11	-	0.7-5.2	0.5-8.6	0.5-1	-	≤LOD[o]	SPE/GC-MS-MS	[46]
Switzerland	-	-	-	-	4.2-11	0.7-1.8	-	2.8	SPE/GC-MS-MS	[28]
Germany	-	-	-	-	0.3-18	0.15-5.2	-	0.1-8.9	SPE/HRGC-NCI-MS	[160]
Germany	27	15	-	-	-	64	-	-	SPE/GC-MS-MS	[18]
Germany[6]	-	-	-	-	9	-	-	<LOD[p]	SPE/GC-MS-MS	[18]

Germany[7]	-	-	-	-	<LOD[q]-22	<LOD[r]-15	-	<LOD[s]-12	SPE/GC-MS	[14]
Germany	-	-	-	-	0.8-4.5	<0.8-0.9	-	-	SPE/GC-MS-MS	[28]

[a]0.3 ng l^{-1}; [b]0.2 ng l^{-1}; [c]0.1 to 1.8 ng l^{-1}; [d]0.4 -0.5 ng l^{-1} influent and 0.2-0.25 ng l^{-1} in the effluent; [e]<0.2 ng l^{-1}; [f]1.6 ng l^{-1}; [g]0.5 ng l^{-1}; [h]1.1 ng l^{-1}; [i]1 ng l^{-1}; [j]1 ng l^{-1}; [k]2 ng l^{-1}; [l]1 ng l^{-1}, [m]1 ng l^{-1}; [n]5 ng l^{-1}; [o]0.5 ng l^{-1}; [p]1 ng l^{-1}; [q]0.7 ng l^{-1}; [r]0.4 ng l^{-1}; [s]0.4 ng l^{-1}; [1]7 STW effluent; [2]trickling filter; [3]activated sludge process; [4]domestic STW; [5]industrial STW; [6]16 STW effluent; [7]18 STW effluent; GC-MS: gas chromatography mass spectrometry; GC-MS-MS: gas chromatography tandem mass spectrometry; LC-MS: liquid chromatography mass spectrometry; LC-MS-MS: liquid chromatography tandem mass spectrometry; LOD: Limit of detection; n.d.: Not detected; SPE: solid phase extraction; ESI: Electrospray Ionisation.

Table 2. Steroid estrogens removal during various STWs treatment processes

Compound	Removal efficiency (%)	Treatment processes	Operations				Sampling period	Season/Air temperature (°C)	Reference
			Flow rate	PE	SRT (d)	HRT (h)			

			(m^3 d^{-1})						
E1	86	ASP (5STWs)	1199000-210000	464000-2020000	2.9-9.8[a]	6.5-12[a]	2001 - 2002	-	[158]
E2	90								
E3	100								
E1	-743[e]	ASP[b]	12300	30000	6	12	2004	Winter[c]	[94]
E2	70								
E1-3S[f]	28								
E2-S[g]	49								
E1	-11[e]	ASP[b]	13400	30000	8.2	10	2004	Summer[d]	[94]
E2	87								
E1-3S	-79[e]								
E2-S	-100[e]								
E1	89.3	ASP[h]	15	-	7-10	0.2	2001	Winter 12-23	[78]
E2	93.4								
E3	91.8								
E1	54.4	ASP[h]	15	-	7-10	0.2	2001	Spring 20-25	[78]
E2	94.7								
E3	99								
E1	59.6	ASP[h]	15	-	7-10	0.2	2001	Summer 25-29	[78]
E2	96.5								
E3	99.2								
E1	72.7	ASP[h]	15	-	7-10	0.2	2001	Autumn 18-23	[78]
E2	94.3								
E3	99.3								
E1	61	ASP[i] (6STWs)	10000-734000	40000-1200000	-	12-14	1999-2000	Autmn to Winter	[39]
E2	87								
E3	95								
EE2	85								
E1	54	ASP	350000	780000	-	-	2002	Spring	[155]

E2	76								
E3	97								
E1	64-75	ASP[k]	145930-148320	284400	6	11	1997	13-19	[72]
E2	92								
E3	n.a[j]								
EE2	n.a[j]								
E1	94-98	ASP[l]	72839-82474	231000	11	18	1997	14-20	[72]
E2	98								
E3	n.d								
EE2	n.d								
E1	66-98	ASP[m]	37247-40661	296400	20	26	1997	15-18	[72]
E2	75-94								
E3	n.d								
EE2	77-98								
E1	n.a[j]	ASP (5STWs)	10368-734400	40000-1.2 x 10^6	15-16	10-14	1999	9-23	[72]
E2	n.a[j]								
E3	n.d-28								
EE2	n.d-2.2								
E1	-54.8[e]-95.1	ASP[n] (9STWs)	5074-585667	24800-1226000	0.9-35.5	11-27	1998	Winter	[36]
E2	39.5-98.3								
E1	-62.4[e]	TF[o]	626000	900000	1.9	6-8	1999	Winter	
E2	-18.5[e]								
E1	82.1	BNR[p]	366898	600000	12.6	23	1998	Winter	[36]
E2	94.7								
E1	66.7-97.8	ASP[q] (3 STWs)	2400-32872	3600-68800	5.5-53	22-61	1998	Autumn-Winter	[36]
E2	82.9-98.8								
E1	46.4-96.1	Lagoon[r]	432-2382	1600-	>150	>150	1998	Autumn	[36]

				6475					
E2	80.5-98.4								
E1	-28.6[e]	Primary sedimentation[s]	2366208	1799000	n.a	3	1999	Winter/Autumn	[36]
E2	-1[e]								
E1	99	ASP+ Oxidation ditch[t]	4800-57000	6500-95000	n.a-30	17.5-22	2000	Summer[t]	[28]
E1	90-99	ASP[u] (3STWs)	1100-8100	11000-30000	8-30	n.a-120	2000-2001	Summer and Autumn[u]	[28]
E1	94-96	ASP[v] (2 STWs)	40000-147000	350000-750000	25-27	8.5-29	1999	Autumn[v]	[28]
E1	89-99	Bioloigcal filter+ASP & Oxidation ditch[w]	4457-61078	15000-240000	n.a	11.6-41	1999	Autumn[w]	[28]
E1	59-85	ASP[x] (3STWs)	8200-45000	27000-110000	3-15	7-19	1999-2000	Autumn[x]	[28]
E1	94-99	ASP[y] (2STWs)	8900-230000	25000-750000	16-19	22-24	1999	Summer[y]	[28]
E1	>99	ASP[z]	6199	71000	7	51	1999	Summer[z]	[28]
E1	Increase-84	Submerged aerated filter and Primary sedimentation[aa]	225000-304400	26600-431600	n.a-20	n.a-4	1999	Autumn[aa]	[28]
16α-hydroxyest	68	ASP[ac]	41200	312000	-	-	1997	Summer (20°C)	[18]

rone									
E1	10[ab]								
E2	64								
EE2	Increase[ab]								
E1	67-83	ASP+ TF[ad]	120096	624000	-	-	1997	Winter (2°C)	[18]
E2	92-99.9								
EE2	64-78								
E1	-80	ASP	-	100000	-	24	2001-2002	Autumn, Winter and Spring	[76]
E2	65								

[a] Supplementary data from [158]; [b] domestic and industrial(10%) wastewater; [c] temperature of influent 16.4°C; [d] temperature of influent 26.7°C; [e] no removal instead increase in concentration; [f] Estrone-3-sulphate; [g] 17β-estradiol-monosulphate; [h] Pilot-scale plant with domestic sewage; [i] HRT 12-14; [j] not applicable value from grab sample therefore % removal not given; [k] Netherland Eindh; [l] Netherland Kral; [m] Netherland West; [n] Canadian mechanical secondary plants B, C, D, E, F, I, O and P; [o] Canadian plant Q – combination trickling filter/solids contact process; [p] Candian plant N – biological nutrient removal; [q] Canadian plants A, G and H - three tertiary plants used sand or granular media filtration (anthracite) as a tertiary treatment process; [r] Canadian lagoon plants J, K, L and M; [s] Canadian plant R – only primary sedimentation with no secondary/tertiary treatment; [t] 2 Belgium plants – ASP and oxidation ditch (effluent temperature 17-20°C); [u] 3 French ASP plants (effluent temperature 12-16°C); [v] 2 Dutch ASP plants (effluent temperature 18-25°C); [w] 2 German STWs (effluent temperature 18-20°C); [x] 3 Swiss ASP plants (effluent temperature 16.4-21°C); [y] 2 Finnish ASP plants (effluent temperature 14-15°C); [z] Swedish ASP plant (effluent temperature 16°C); [aa] 2 Norwegian submerged aerated filter and a primary sedimentation plant (effluent temperature 12-13°C); [ab] values estimated from [18]; [ac] German municipal STW; [ad] Brazilian municipal STW; n.d: not determined; n.a: not applicable.

Table 3. Equipment and O&M costs for EDC removal options

Process/Technology	Estimated Equipment cost* ($/gal)	Estimated O&M cost ($/1000gal)
MBR	1.00-2.50	**
IFAS	0.20-0.30	**
Peroxide/Ozone	0.40-0.80	0.40-0.80
UV/Peroxide	0.40-0.60	0.30-0.50
MF/RO	1.65-3.74	0.6-1.00
MF/RO followed by UV/Peroxide	2.05-4.34	0.90-1.50

Adapted from Scruggs et al. [136]

* Does not include cost of construction

** Separate costs not determined