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Occurrence and fate of estrone, 17β-estradiol and 17α-ethynylestradiol in STPs for domestic wastewater

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Abstract

Estrone (E1), 17β-estradiol (E2) and 17α-ethynylestradiol (EE2) discharged from sewage treatment plants (STPs) into surface waters, are seen as a threat effecting aquatic life by its estrogenic character. Therefore, much research is conducted on the fate and removal of these compounds. Since these compounds are present in influents and effluents in the ng/l range, methods for detection deserve special attention. Most important processes that play a role in the removal of estrogens are: adsorption, aerobic degradation, anaerobic degradation, anoxic biodegradation and photolytic degradation. Halflifes tend to vary and are remarkably shorter when low initial concentrations are applied. In general anaerobic conditions result in longer halflifes then aerobic conditions. EE2 shows far most persistence of the compounds, thereby also the estrogenic effect *in vitro* is about 2–3-fold higher compared to E2. The three compounds show a higher affinity to sorb to sludge compared to other tested adsorption materials like sediment. Aerobic degradation is far the most efficient in removing these compounds, but adsorption seems to play a significant role in retaining the estrogens inside full-scale STPs. Removal rates in full scale plants depend on the HRT, SRT and loading rates, but lack of information on the exact dependency so far prevents an optimal design able to fully eliminate estrogens from wastewater.

1. Introduction

Due to the proven estrogenic effect of sewage treatment plant (STP) effluents much research is directed towards the occurrence and fate of estrogenic compounds in wastewater. Three sterols, the natural hormones 17β-estradiol (E2) and estrone (E1) and the synthetic hormone 17α-ethynylestradiol (EE2), were isolated from effluents of domestic STPs and identified as a prime contributor to its estrogenic character (Desbrow et al. 1998; Routledge et al. 1998; Körner et al. 2001; Onda et al. 2003). Xeno-estrogens, which are non-steroid compounds with estrogenic potency, including pesticides, plasticizers (e.g., bisphenol-A), polychlorinated biphenyls, alkylphenols, phthalates and brominated flame-retardants, contribute to approximately 1–4% of the total estrogenic activity of wastewater (Körner et al. 2000). Alkylphenols contribute less than 0.5% of the total estrogen equivalents in samples of STP effluents in south central Michigan (Snyder et al. 2001). Despite their low contribution in practice, a combination of multiple xeno-estrogens can still lead to a dramatic enhancement of estrogenity (Rajapakse et al. 2002). The reason for the estrogenic activity of numerous substances is the relatively low specificity of the human estrogen

receptor because the binding pocket is nearly twice as large as the molecular volume of E2 (Brzozowski et al. 1997).

This review emphasises the behaviour of E1, E2 and EE2 in STPs, to evaluate current knowledge and to point out where information is lacking. At first the amounts expected to enter STPs within wastewater are outlined. Then the necessity of gaining knowledge on this subject is outlined by a description of the environmental consequences and prevalence, followed by a brief description of analytical procedures. After that different removal processes and the importance of these particular processes for the removal of E1, E2 and EE2 are described. Then the removal efficiencies in current STPs and eventual additional treatment systems are reported. Finally a conclusion is drawn on current knowledge and suggestions for further research are identified.

2. Excretion of estrogens

Estrogens influence growth, development, differentiation and function of peripheral tissues of the female and male reproductive system, such as the mammary gland, uterus, vagina, ovary, testis, epididymis and prostate; and they play an important role in bone maintenance, the cardiovascular system and the central nervous system (Shimada et al. 2001). Estrogens are produced by human glands, mainly by ovaries and testis, and are excreted within urine and faeces. The synthetic EE2 is the main estrogen used in oral contraceptives, which is the most prescribed drug world-wide (Williams & Stancel 1996). Estrogens are metabolised in the liver, where they are enzymatically mediated and conjugated with either sulphate or glucuronide esters to the hydroxyl groups in the C3- and C7-position of the basic steroid structure (Williams & Stancel 1996). Theoretically there are eight different possible conjugates for E2 and EE2, as they have two hydroxyl groups. E1 has only two possible conjugates (Kjølholt et al. 2004). Conjugation increases the water solubility, which eases excretion, but also makes them more mobile in the environment compared to free hormones. Conjugated hormones that have a group on the C3 position are biologically inactive (Ingerslev & Halling-Sorensen 2003) and do not exhibit estrogenic properties. Estrone is excreted as sulphate- rather than glucuronide conjugate as has been found in urine from pregnant women (Andreolini et al. 1987). In human urines, the glucuronides are dominant (male 85%, female 65%), against sulphates (male 15%, female 35%) (Matsuda et al. 2001). Natural hormones are mainly excreted in urine and only a very small amount is excreted in faeces in a mainly unconjugated form. The reason for the estrogens being unconjugated in faeces is the ability of bacteria, for instance *E.coli*, to produce the enzyme β-glucuronidase, which can hydrolyse glucuronide conjugates back to their original form (Ternes et al. 1999a; Legler 2001).

According to Williams and Stancel (1996), the total daily excretion rate of natural estrogens ranges from 10 to 100 μg for women, 5–10 μg for women after the menopause and 2–25 μg for men. Average excretion values from a study amongst female inhabitants of a Roman condomium were 32, 14 and 106 μg/day of conjugated E1, E2 and E3, respectively (D'Ascenzo et al. 2003). According to Adlercreutz et al. (1986) women can excrete with urine about 7 μg of E1 2.4 μg of E2, and 4.6 μg of E3 of unconjugated forms daily. Approximately 0.4 μg E2, 1.25 μg of E3 and 0.5 μg E1 is eliminated in faeces per day (Adlercreutz et al. 1994). Fotsis et al. (1980) reported a daily excretion in urine of unconjugated forms as 3.0 μg of E2, 8.0 μg of E1 and 4.8 μg of E3. Calculations for the percentage contribution to the total excretion of both conjugated and unconjugated natural estrogens and the synthetic EE2, are shown in Figure 1, illustrating that pregnant women contribute the most estrogens to the total excreted amount.

The average daily dose of the synthetic hormone used in the contraceptive pill is 35 μg EE2, taken during 21 days of a 28 day period (Katzung 1995). Up to 80% of the EE2 digested is excreted as unmetabolized conjugates (Ranney 1977; Maggs et al. 1983). Of the daily dose, 22–50% of EE2 is excreted in urine of which about 64% is conjugated and approximately 30% is excreted in faeces (Reed et al. 1972). The oral bioavailability of EE2 is about 42% due to an extensive first-pass metabolism in the intestinal wall and liver (Weber et al. 1996). More than 30% of EE2 is sulphated, which accounts for approximately 60% of the first-pass metabolism (Back et al. 1979, 1982). Only 1–2% of the administered EE2 has been

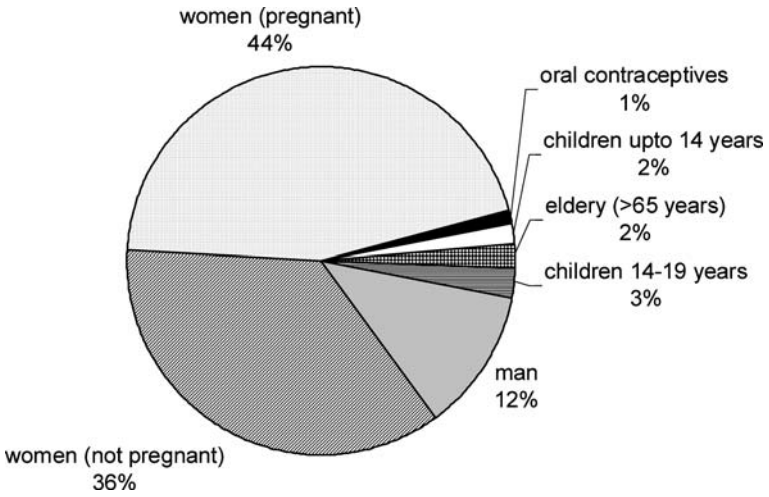


Figure 1. Contribution of different groups of the Dutch population to the amount of total excreted natural estrogens and 17α-ethynyestradiol; based of the total population figures of 2001 (CBS 2002).

found to be de-ethynylated and transformed to E1, E2 or E3 (Ranney 1977). In 2001, 43% of the female Dutch population in the age of 16–49 years used oral contraception (CBS 2002). The contribution of EE2 to the total amount of excreted estrogens is only about 1% (Figure 1), but this compound is considerably more persistent in STPs compared to the natural hormones (Ternes et al. 1999a, b). Due to the introduction of the ethynyl-group, the ring becomes extremely stabile against oxidation. Thereby EE2 showed the highest estrogenic potency *in vitro* tests, the potency compared to the other estrogens can be expressed as EE2 > E2 > E1 (Larsson et al. 1999). It has also been showed that *in vivo* tests in fish, EE2 was 11–130 times more potent than E2, while E2 was 2.3–3.2 times more potent than E1 (Legler 2001; Metcalfe et al. 2001; Thorpe et al. 2003). The amount of E2 used for pharmaceutical purposes contributes less than 5% compared with the natural E2 excretion (Christensen 1998).

Estimations of the maximum concentration of natural estrogens present in wastewater are about 1 μg/l and for the synthetic EE2 about 13.4 ng/l (Blok & Wösten 2000). This calculation is based on a wastewater production of about 200 l per person per day.

Measurements in municipal influents are generally lower than these estimated values, for example in the Netherlands, values were ranging from 20 to 130 ng/l for E1, from 17 to 150 ng/l for E2 and <0.3–5.9 ng/l for EE2 (Vethaak et al. 2002). The samples were filtered first, so only the hormones in the liquid phase were measured, and no deconjugation step was applied, although a considerable amount of conjugated estrogens can be present in influents (58% of total E1 and E2 and 26% of EE2) (Adler 2001).

3. Environmental consequences and prevalence

Estrogens present in discharged domestic effluents represent the most significant estrogenic input to the aquatic environment and serve as important point sources, especially in densely populated areas (Belfroid et al. 1999b; Ternes et al. 1999b). Figure 2 illustrates this pathway. Many scientific groups worldwide have stated the hypothesis of an association between increased estrogens in the environment and the adverse trends in reproductive health, and the prevalence of cancer in endocrine sensitive tissues (e.g., breast, prostate, testis) (Bosland et al. 2002; USDHHS 2002). For humans, a causal relation has not been firmly established (Safe 2000). However, for fish there are direct correlations with the discharge of STP effluents in surface water and the feminisation of male fish and early life exposure can affect sex ratio by increasing the female phenotype (Jones et al. 2000; Vethaak et al. 2002). Concentrations as low as 0.5 ng/l of

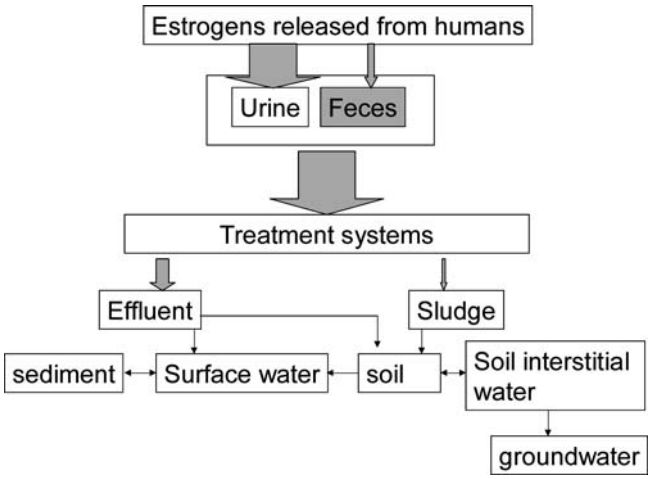


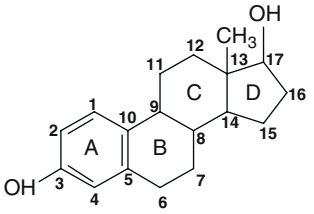
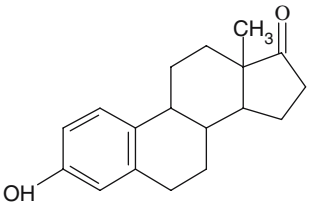
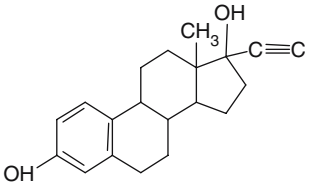
Figure 2. Pathway of estrogens from release by humans to ending up in the different environmental compartments.

EE2 leads to an induction of vitellogenin, a protein responsible for the formation of oocytes, in male trout after 10 days exposure (Purdom et al. 1994; Hansen et al. 1998). The predicted no effect concentration is set on 0.1 ng/l for EE2 and 1 ng/l for E2 (EA 2002). Beside the direct effects on the aquatic environment, there is a suspected bioaccumulation via the food chain for all three compounds (Lai et al. 2002; Takahashi et al. 2003; Gomes et al. 2004). There is even an effect observed on the plant alfalfa; irrigation with wastewater led to an elevated level of phytoestrogens (Shore et al. 1995). Adding E1 and E2 to irrigation water in a concentration range of 5–500 ng/l did increase growth, while higher concentrations in the range 50–500 μg/l inhibited growth (Shore et al. 1992).

Common median values for estrogens measured in STP effluents are ranging from 1 to 11 ng/l for E1, E2 and EE2 in Sweden (Larsson et al. 1999), Germany and Canada (Ternes et al. 1999b), Southwestern Germany (Spengler et al. 2001), UK (Desbrow et al. 1998), Italy (Baronti et al. 2000) and the Netherlands (Belfroid et al. 1999b). Values for individual measurements may be higher, the maximal value measured for E1 was 76 ng/l in the UK (Desbrow et al. 1998) 64 ng/l for E2 and 42 ng/l for EE2 both measured in Canada (Ternes et al. 1999b). In river water samples from Italy, values for E1, E2 and EE2 were ranging from 0.04 to 1.5 ng/l (Baronti et al. 2000). From a study conducted in Spain, 8.0 ng/l of E1 was detected in river water and up to 22.8 μg/kg in sediment (Petrovic et al. 2002). In the UK values in river water were ranging from <0.4 to 12.2 ng/l for E1, from <0.4 to 4.3 ng/l for E2 and from <0.4 to 3.4 ng/l for EE2 (Williams et al. 2003). Measurements done in two rivers gave a similar estrogenity of about 1.4–2.9 ng E2 equivalents/l, while measurements were near the limits of detection 1 km upstream and downstream of the STP. Levels in the sediment were between 21.3 and 29.9 ng E2 equivalents/kg both at upstream and downstream sites (Peck et al. 2004). This suggests that riverine sediments are a sink and potential source of estrogenic contaminants. In the Netherlands E1 was detected from <0.3 to 7.2 ng/l in surface water, E2 from <0.8 to 1.0 ng/l and EE2 from <0.3 to 0.4 ng/l (Vethaak et al. 2002). Kuch and Balsmiter (2001) have detected E1, E2 and EE2 in tap water originating from three drinking water plants in south of Germany up to 0.6, 2.1 and 0.5 ng/l, respectively (Kuch & Ballschmiter 2001). E1, E2 and EE2 were not detected in tap water by other researchers (Stumpf et al. 1996; Fawell et al. 2001).

The fate of E1, E2 and EE2 in STPs is related to their characteristics, which are summarised in Table 1, which also gives the structural formulas. Many different log K_{ow} values have been reported; some of them have been calculated. Since the K_{ow} is approximately 4, an important amount of these compounds are expected to bind

Table 1. Chemical structure and physiochemical properties of E2, E1 and EE2

Name	17β-Estradiol (E2)	Estrone (E1)	17α-Ethynylestradiol (EE2)
Structure			
Formula	$C_{18}H_{24}O_2$	$C_{18}H_{22}O_2$	$C_{20}H_{24}O_2$
Molecular weight (g)	272.39	270.37	296.40
Aqueous solubility at 20 °C (mg/l)	13[a]; 12.96[b]	13[a]; 12.42[b],12.4[c]	4.8[a]; 4.83[b], 3.8–4.5[d]; 4.7[e]
Henry's law constant (atm m^3mol^{-1}; at 25 °C)	3.64E−011[f] 6.22E-012[g]	3.8E−010[f] 6.12E-012[g]	7.94E−012[f] 3.75E-012[g]
Log K_{ow}	3.94[f]; 4.01[h]; 3.10[i]	3.4[a]; 3.43[f]; 3.13[h]	4.1[a]; 4.15[f]; 3.67[h]; 3.9[i]
Size (nm)[j]	0.398	0.396	0.416
pK_a	10.46 ± 0.03[k1]; 10.4[l]	10.34 ± 0.05[k]	10.40 ± 0.0[k]; 10.7[l]

[a]In double distilled water (Lai et al. 2000).
[b]Tabak et al. (1981).
[c]Jürgens et al. (1999).
[d]In wastewater (Norpoth et al. 1973).
[e]In wastewater (Tabak et al. 1970).
[f]Calculated (SRC 2003).
[g]Lai et al. (2002).
[h]Hansch et al. (1995).
[i]Jürgens et al. (2002) and Holthaus et al. (2002).
[j]Calculated according (Worch 1993) and Stokes Einstein (Schäfer et al. 2003).
[k](Hurwitz and Liu 1977) [k1] value for 17a-estrdaiol.
[l]Clara et al. (2004b).

to sludge, soil and sediment. The pK_a is around 10.4 for all three compounds. Solubility for EE2 is lower in wastewater compared to pure water (Norpoth et al. 1973). Synthetic hormones are more stable in water than natural hormones (Aherne et al. 1985). This is also supported by the ratio between EE2 and E2 in surface water, which is higher than the theoretical ratio based on human excretion, indicating a faster degradation of the natural estrogens (Larsson et al. 1999).

4. Analytical procedures

Detection and measurement of steroid estrogens in sewage influent and effluent is a difficult and expensive procedure, and is still a long way from becoming a routine analysis (Johnson et al. 2000). As concentrations of estrogens in wastewater are generally low, a concentration step is required. In most cases, solid phase extraction (SPE) is used, applying either SBD-XC or C18 disks or cartridges (Xiao et al. 2001), but also liquid–liquid extraction (Mol et al. 2000) and freeze-drying are options (López de Alda & Barcelo 2001; Khan et al. 2002; Ternes et al. 2002). The latter is usually used for the analysis in non-watery matrices. For extraction, different solvents have been used, most commonly used are methanol (Kelly 2000), mixtures of acetone/methanol (Ternes et al. 2002) and acetonitrile. The sample is dried and reconstituted in for example methanol, acetone or acetonitrile (López de Alda & Barcelo 2000). Before or after this concentration step, glucuronide and sulphatase enzymes can be added to convert eventual conjugated hormones into unconjugated hormones (Belfroid et al. 1999b; Huang & Sedlak 2001; Legler et al. 2002). Cleaning up by a silica-column or a HPLC can be applied. Latter is used

to separate different hormones as they have a different residence time in the column (Belfroid et al. 1999b; Huang & Sedlak 2001; Williams et al. 2003).

Concentrated samples can be analysed using different types of quantification techniques, the following can be mentioned: Gas Chromatography with Mass Spectrometry (GC/MS) (Mol et al. 2000; Jeannot et al. 2002), Gas Chromatography with tandem Mass Spectrometry (GC/MS/MS) (Ternes et al. 2002; Williams et al. 2003), Gas Chromatography Negative-Ion Chemical-Ionization Mass Spectrometry (GC/MS/NICI) (Nakamura et al. 2001), Gas Chromatography with Mass Spectrometry and Electron Impact (GC/MS/EI) (Nakamura et al. 2001), Gas Chromatography Negative Chemical Ionisation Mass Spectrometry (GC/NCI/MS) (Xiao et al. 2001), Liquid Chromatography with Mass Spectrometry (LC/MS) (Jeannot et al. 2002), Liquid Chromatography with tandem Mass Spectrometry (LC/MS/MS) (Ingrand et al. 2003; Isobe et al. 2003), Liquid chromatography electrospray ionisation tandem mass spectrometry (LC/ESI/MS/MS) (Baronti et al. 2000), Liquid Chromatography with Diode Array Detector (LC-DAD) (Braga et al. 2001), Liquid Chromatography with Diode Array Detector and Mass Spectrometry (LC/DAD/MS) (López de Alda & Barcelo 2001), High Resolution Gas Chromatography with Mass Spectrometry operating in Single-Ion Monitoring (HRGC/MS/SIM) (Kuch & Ballschmiter 2000), High Performance Liquid Chromatography (HPLC) (Snyder et al. 1999), immunoassay (Khan et al. 2002) or bioassay (*in vitro* and *in vivo*) (Folmar et al. 2002; Rutishauser et al. 2004).

A pre-derivatisation step is demanded for GC in order to make the compounds more volatile. For this purpose mixtures of *N*-methyl-*N*-tert.-butyldimethylsilyltrifluoracetamide (MTBSTFA) containing 1% tert.-butyldimethylchlorosilane (TBDMCS) (Kelly 2000; Williams et al. 2003), pentafluorobenzylbromide and trimethylsilimidazole (Braga et al. 2001), or heptafluorobutyric anhydride (Huang & Sedlak 2001) are used. Further information can be obtained from a report from the Danish Environmental Protection Agency on the evaluation of analytical chemical methods for detection of estrogens in the environment (Ingerslev and Halling-Sorensen 2003).

4.1. *Immunoassay*

Two immunoassay techniques are the enzyme-linked immunosorbent assay (ELISA) and radio-immunoassay (RIA). The technique is based on the reaction with antibodies and although antibodies are biologically derived reagents, immunoassays are physical assays based on the law of mass action (Bunce et al. 2000). The antigen, estrogens in a sample, will bind to the inner surface of a well or tube coated with a protein antibody. An amount of an estrogen labelled with a colouring enzyme, or radio labelled is added. When a lot of estrogens were present in the sample, less binding places are available for the labelled estrogens. The excess estrogens are washed out and a substrate is added to the antibody tubes and the amount of bound antigen-enzyme conjugates develops a colour (Wako 2002). A higher concentration of estrogens in the sample, leads to less antigen-enzyme conjugates and a lighter colour. In case of radio labelled estrogens, scintillication is used. Disadvantages of this technique include cross-reaction and matrix effects (Voulvoulis & Scrimshaw 2003).

4.2. *Bioassay*

Competitive ligand binding assays, cell proliferation assays and *in vitro* gene expression assays are the most common *in vitro* approaches for estrogenic compounds (Snyder et al. 2000). A bioassay makes use of common mechanisms of action as occur in vertebrates. Estrogens are transported through the blood mainly bound to sex hormone binding globulins; free estrogens can exert their action through diffusing through cell membranes and binding estrogen receptors (ER) (Legler 2002). ERs are found in many tissues, including reproductive organs and accessory sex organs, brain, bone and liver. After dimerization of two ER-ligand complexes, binding to estrogen response elements (ERE) of genes on the DNA in the nucleus takes place. After transcription, mRNA is translated into protein by ribosomes.

Competitive ligand binding assays are based on the fact that estrogen agonists and estrogen antagonists are binding to the ER and will both lead to positive responses (Bunce et al. 2000). This assay investigates the ability of compounds

to bind *in vitro* to the ER, thereby displacing (radioactive) labelled E2 from the ER. The amount of radioligand bound in the control compared to amount radioligand bound in the sample to be measured lead to a quantification of the amount of estrogens present in the sample. The greatest limitation of these assays is that although compounds may bind to the receptor, the tests do not distinguish between agonistic and antagonistic effects (Zacharewski 1997, 1998).

Cell proliferation techniques, also known as ''E-screen'', are based on human-derived cell lines and utilize a number of end points to measure the cell proliferation induced through exposure to estrogenic compounds (Voulvoulis & Scrimshaw 2003). A major problem is the reproducibility, since many different cell lines are in use.

The *in vitro* gene expression assays, also called recombinant receptor-receptor assays, are undertaken with genetically engineered mammalian cells or strains of yeast. The cells have been transfected or transformed with recombinant DNA, so that exposure to estrogens not only lead to the production of the protein, but also to the production of, for example, the firefly enzyme luciferase (in ER-Calux method, with human breast cancer cells, (Legler et al. 2002) or β-galactosidase (in YES-assay with yeast cells, (Murk et al. 2002)). Luciferase can be measured by adding the substrate luciferin, resulting in a light producing reaction and β-galactosidase change the assay medium from yellow to red. The ER-calux assay, appeared to be the most sensitive, in which the detection limit was 0.5 pM, followed by the YES assay with a limit of 10 pM for E2 and the ER-binding assay with 1000 pM (Murk et al. 2002).

Although *in vitro* assays are an attractive option, because they are rapid, inexpensive and fairly reproducible, they may miss effects that would take place only in whole organisms (Snyder et al. 2000). This makes *in vivo* assays necessary for the evaluation of impacts on the endocrine system as a whole (Voulvoulis & Scrimshaw 2003).

4.3. In vivo

For *in vivo* experiments in the aquatic environment, fish are often used. In example, adult male rainbow trout and adult roach were exposed to STP effluent levels of estrogens and plasma levels of vitellogenin were determined to measure an estrogenic response (Purdom et al. 1994; Routledge et al. 1998; Jones et al. 2000; Folmar et al. 2002). The presence of vitellogenin in male fish is a useful biomarker to identify estrogenic activity in sewage effluents (Hansen et al. 1998; Jones et al. 2000; Solé et al. 2001; Hennies et al. 2003). Besides vitellogenin production, impact on condition and gonadal growth was investigated in roach after long-term exposure to STP effluent (Rodgers-Gray et al. 2000).

4.4. *Validation*

Different studies are performed on the validation of methods. It was concluded that compared to GC/MS/MS, the immunoassay (ELISA) technique has lower detection limits, requires smaller concentration factors, and is less susceptible to matrix interference (Huang & Sedlak 2001). The theoretical values of estrogenic activity calculated from the concentrations of each estrogen by LC-MS/MS in treated wastewater were found to correlate with values of estrogenic activity measured by yeast estrogen screen assay (Onda et al. 2003).

Storage of samples is an important issue as severe losses of estrogens were observed during storage of bottled river water after 7 days at 4 °C. The best way to store samples is on carbograph material in a cartridge (after SPE) and stored at −18 °C (Baronti et al. 2000).

The different techniques used for determination can lead to difficulties in comparing results, as they have different precision and detection limits. It is also not always clear whether a pre-deconjugated step was applied during the measurements in different researches. Also the method of pre-concentrating samples from different matrices seem to have a large impact on the recovery of estrogens and thus on the amount finally measured.

5. Fate in biological STPs

Processes playing a role in the removal of estrogens from the aquatic phase are: adsorption, aerobic biodegradation, anaerobic biodegradation, anoxic biodegradation, and photolytic degradation. Volatisation is not expected to play

a significant role in removal of E2, E1 and EE2, since compounds with a Henry's law constant (H_c) lower than 1E−04 and an H_c/K_{ow} ratio lower than 1E−09 do exhibit a low volatisation potential (Rogers 1996) (Table 1). How these processes can be interlinked with each other for the three estrogens is illustrated in Figures 3 and 4, which are adopted from a model used to predict the behaviour of the different estrogens in STPs, developed by Joss et al. (2004). In these figures it is shown that estrogens are present in the water bulk in unconjugated forms, where they are biodegraded. Under aerobic or anoxic conditions, E2 will be first oxidised to E1, which is further oxidised to unknown metabolites and finally to CO_2 and water. EE2 is oxidised to unknown metabolites and also finally to CO_2 and water. Under anaerobic conditions, theoretically E1 can be reduced to E2. More information on adsorption and degradation under different environmental conditions is outlined below.

5.1. *Adsorption*

Adsorption to organic material is expected to play a significant role in reducing concentrations in the aqueous phase. The octanol-water (K_{ow}) and organic carbon (K_{oc}) partition coefficients

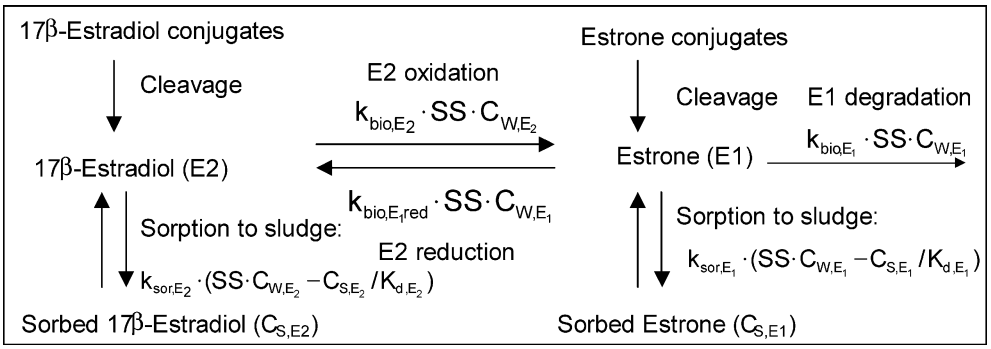


Figure 3. Schematic review of behaviour of estrone (E1) and 17β-estradiol (E2) in STPs, adopted from Joss et al. (2004), Abbreviations: k_{bio} and k_{sor} are pseudo-first-order reaction rate constants; SS suspended solids; C_w bulk soluble concentration; C_s sorbed concentration per reactor volume; K_d sorption coefficient.

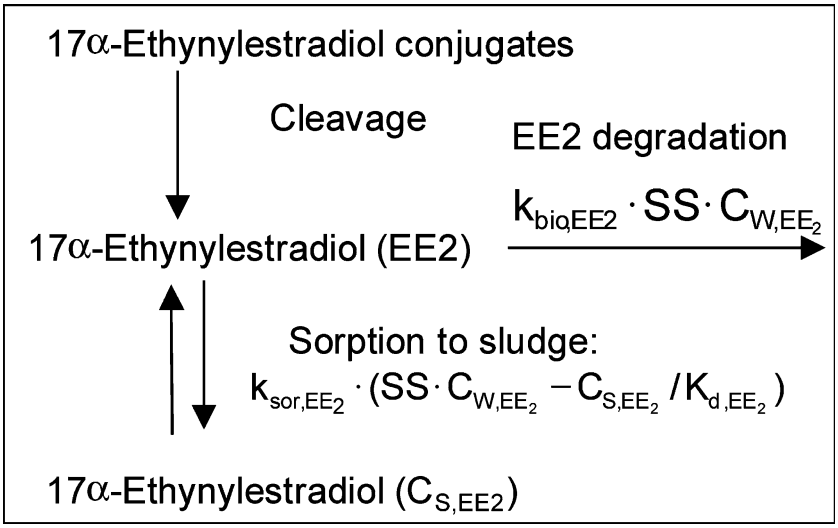


Figure 4. Schematic review of behaviour of 17α-ethynylestradiol (EE2) in STPs, adopted from Joss et al. (2004) (Abbreviations see Figure 3).

are indicators used for adsorption to organic material. A relation between log K_{oc}, water solubility and log K_{ow} has been reported according to Equations 1 and 2, in which S is the water solubility (mg/l) (Means et al. 1982).

$$\log K_{oc} = 0.686 \log S + 4.273 \quad (1)$$

$$\log K_{oc} = \log K_{ow} - 0.317 \quad (2)$$

Estrogens are hydrophobic organic compounds of low volatility, with log K_{ow} values of 3.43 for E1, 3.94 for E2 and 4.15 for EE2 and a solubility in water at 20 °C of 13 mg/l for E1 and E2 and 4.8 mg/l for EE2 (Lai et al. 2000). As an indication, compounds with a log K_{ow} below 2.5 exhibit a low sorption potential, between 2.5 and 4.0 a medium sorption potential and higher than 4.0 a high sorption potential (Rogers 1996). Using a model to predict the fate of chemicals in STPs gives a 46% loss onto discharged sludge for a substance with log K_{ow} 4 (Struijs et al. 1991; Panter et al. 1999). Applying the same model gives <10% loss on discharged sludge for a substance with log K_{ow} 3 and >75% for a substance with log K_{ow} 5. Since the reported log K_{ow} values for E1, E2 and EE2 are varying in between 3 and 4 it cannot be fully predicted how much is eventually discharged with the sludge. Further in the review it is shown, that this 46% is an overestimate and only at most 5% of E1, E2 and EE2 finally is discharged with the sludge.

Sorption can be described using a Freundlich isotherm (Equation 3). In which C_s is the concentration on sorption material at equilibrium, C_w concentration in the water phase and K_f and $1/n$ are the sorption coefficient and constant. When the concentration is really low, and the binding places are not fully occupied ($1/n = 1$), the relation with the sorption coefficient K_d (l/kg) is linear (Equation 4).

$$C_s = K_f C_w^{1/n} \quad (3)$$

$$C_s = K_d C_w \quad (4)$$

Several tests were conducted to study the adsorption behaviour of estrogens to activated sludge, anaerobic sludge, sediments, soils or other organic materials. From research on the sorption on sediments, three sorption phases are distinguished, a rapid sorption between 0 and 0.5 h, followed by a period of slower sorption up to 1 h, after which a steady decrease in sorption, explained by an increase in dissolved organic matter in the water phase (Lai et al. 2000). For activated carbon an equilibrium for E2 was reached after 50–180 min (Fuerhacker et al. 2001). From adsorption isotherms by Jürgens et al. (1999) an equilibrium is nearly reached after two days in river sediments, but after 5 days the amount on the sorbent is still increasing. Even a final equilibrium of 50 days have been reported in river water sediments (Bowman et al. 2003). The time to reach an equilibrium is obviously related to the type of sorption material, as well as the test conditions. It can be said that after a shorter period of several hours or days, more than 90% of the equilibrium concentration is already reached.

5.1.1. *Sorption on sediments*

On sediments, for E1, K_f was 54 l/Kg (K_d 8 l/kg), for E2 36 l/kg (K_d 4 l/kg) and for EE2 52 l/kg (K_d 5 l/kg) and $1/n$ was for E1 0.73, E2 0.67 and EE2 0.83 (Lai et al. 2000). A sorption constant below 1 indicates that sorption approached a limit and the binding sites have been occupied. The limited amount of binding sites is illustrated by adding the superhydrophobic compound estradiol valerate ($K_{ow} = 6.41$) which showed suppressed sorption of the other estrogens, suggesting competition for binding sites (Lai et al. 2000). Sorption increased with increasing salinity by adding NaCl, leading to aggregation and flocculation. Holthaus et al. (2002) conducted adsorption experiments in bed sediments under anaerobic conditions in the lower concentration range and therefore the simplified isotherm was used, (Equation 4), to calculate the K_d value for E2 and EE2 on different bed sediments. Around 80–90% of the equilibrium was achieved within 1 day, but a complete equilibrium was only achieved after 2 days. EE2 showed a greater affinity to the bed sediments in all cases, with sorption K_d values 1.6–3.1-fold higher than those determined for E2. The K_d values were ranging from 4 to 72 l/kg for E2 and from 8 to 121 l/kg for EE2. In general, higher K_d values were associated with smaller particle size and higher organic carbon content in bed sediments.

5.1.2. *Sorption on other organic materials*
Sorption of E2 and EE2 was investigated onto several commercially available organic colloidal compounds including polysaccharides and humic substances, which are found in typical streams and rivers (Yamamoto & Liljestrand 2003). K_{oc} values were calculated with Equation 5, in which F is the concentration in solution and F_0 the concentration in absence of organic colloids.

$$F_0/F = 1 + K_{oc}[\text{Organic collid(kgTOC/l)}] \quad (5)$$

Yanamoto and Liljestrand (2003) found sorption of E2 and EE2 to be highest on tannic acid, with a log K_{oc} of 5.28 and 5.22, respectively, and the lowest for the polysaccharide algic acid, 2.62 and 2.53, respectively. The values for some humic acids were somewhat lower than for tannic acid. So binding is better onto substances containing aromatic rings, caused by interaction between π-electrons. The poor relationship between log K_{ow} and log K_{oc} suggests significant contributions of other sorption mechanisms than hydrophobic interaction. In a pH range of 4–9.5 at a constant ionic strength of 0.02 M and for different ionic strengths ranging from 0.01 to 1 M at a constant pH of 7, no significant differences in K_{oc} were observed. It was concluded from this research that in typical natural waters of 5 mgTOC/l, approximately 15–50% of the estrogens are bound.

5.1.3. *Sorption on sludge*
For sorption of E1 and E2 to activated sludge the highest percentage adsorbed was 23% at pH 8 and 55% at pH 2 (Jensen & Schäfer 2001). At a concentration range of 5–500 ng/l radio labelled E1 and E2, adsorption to activated sludge is linear, indicating adsorption sites are in excess (Schäfer et al. 2002b). The adsorption percentage is depending on the sludge concentration, as approximately 15% of E1 was adsorbed at approximately 2 g sludge/l and 30% was adsorbed at 8 g sludge/l.

In wastewater, containing 128 mg SS/l, spiked with radio labelled E2 to a concentration of 50 ng/l, 86% of the radioactivity remained in the liquid phase after 24 h (Fürhacker et al. 1999). This research is illustrating circumstances in the sewer system, as raw municipal wastewater was spiked, with no addition of activated sludge, and incubation was without aeration. In a test with activated sludge in a concentration of 2–5 g/l, only 20% of labelled EE2 remained in the aqueous phase after one hour, when 20% mineralisation was observed, concluding that 60% can be bound to the sludge (Layton et al. 2000). During a biological oxygen demand (BOD) test, 28% of E2 and 68% of EE2 was calculated to be sorbed to sludge after 3 h incubation, which is greater than 20% and therefore considered of significance (Kozak et al. 2001).

Joss et al. (2004) has used a dynamic sorption kinetic model to calculate the flux from the soluble phase to the solid sludge particles resulting in Equation 6:

$$r = k_{sor} \cdot \left(SS \cdot C_{W,bulk} \cdot \frac{C_{S,reactor}}{K_d} \right) \quad (6)$$

in which r is the flux (g/l/day), k_{sor} is the pseudo-first-order sorption rate constant (l/gSS/day], $C_{W,bulk}$ is the soluble estrogen concentration in the bulk liquid phase (g/l), $C_{S,reactor}$ is the sorbed estrogen amount per reactor volume (g/l) and K_d is the distribution coefficient. Using this model for the results of an STP in Wiesbaden (Andersen et al. 2003), it gives a $k_{E1,sor}$ of 4100 ± 800 l/kg/day and a $K_{d,E1}$ of 900 ± 100 l/kg (Joss et al. 2004). This could not be calculated for E2 and EE2, as the data were too close to their analytical limit of quantification. K_f and K_d values for de-activated sludge from Ega STP, Lundtofte STP and Austrian plant are shown in Table 2. Sludge was deactivated by freeze-drying, followed by sterilization for 2 h at 103 °C in the first two cases. This pre-treatment might have had some effects on the sorption capability of the sludge, resulting in high standard deviations and lower obtained adsorption coefficients than the calculated value of 900 l/kg for E1. In general, K_f and K_d values are highest for sludge in comparison with sediments and other organic materials. For the Austrian plant mercury sulphate was used for de-activation, which showed to have no effect on the adsorption capacity, as the K_f and K_d values for both activated and inactivated sludge gave the same values (Clara et al. 2004b). Clara et al. (2004b) also researched the influence of the pH on the adsorption and found that near the pK_a value (pH > 9) both E2 and EE2 started to desorb as a result of increased solubility of these compounds in the dissociated form.

Table 2. K_f and K_d values for batch experiments with sludge from Ega and Lundtofte STP (Kjølholt et al. 2004)

Sludge origin	Compound	K_f (l/kg)	K_d (l/kg)
Ega STP	E1	822 (918)	249
	E2	594 (281)	236
	EE2	267 (257)	436
Lundtofte STP	E1	89 (105)	570
	E2	1106 (627)	360
	EE2	383 (245)	459
STP in Austria; inactivated sludge (Hg_2SO_4, 200 g/l)	E2	620	692
	EE2	480	692
STP in Austria; activated sludge	EE2	480	692

Standard deviations in brackets and with activated and inactivated sludge from an Austrian STP (Clara et al. 2004b).

The information on the adsorption on anaerobic sludge is scarce. (Pakert et al. 2003) found in batch tests with anaerobic sludge with a TSS content of 30 g/l, 75% of E2, 85% of E1 and 90% of EE2 was adsorbed. (Kunst et al. 2002) reported values adsorbed to anaerobic sludge during sludge treatment of 3–115 μg/kg TS for E2 and 3–330 μg/kg TS for E1. EE2 was not detected.

5.2. *Aerobic biodegradation*

Estrogen conjugates are cleaved into their active forms, as found in batch experiments using activated sludge (Ternes et al. 1999a). The initial transformation of 17β-estradiol-conjugates to an estrogenically active product occurs more rapidly than degradative loss (Panter et al. 1999). Deconjugation of glucuronide conjugates is expected to already take place in sewer systems, while cleavage of the sulphuric conjugates, (which need arylsulphatase for cleavage), will only happen in STPs as this demands more specialistic microorganisms (Baronti et al. 2000). This is confirmed with measurements at the STP entrance, where free estrogens and sulphated estrogens were the dominant species (D'Ascenzo et al. 2003). Also in lab scale experiments with wastewater and the addition of both types of conjugates, it took approximately 3 days for the sulphate conjugates, against 7 h for the glucuronide conjugates to reach half the initial concentration (D'Ascenzo et al. 2003).

In aerobic batch experiments it was shown that after a period of 1–3 h, more than 95% of E2 was oxidised to E1 (Ternes et al. 1999a). In the same experimental set up, EE2 appeared to be stable. Also Norpoth et al. (1973) found no degradation of EE2 in activated sludge after an incubation time of five days. The findings for the conversion of E2 to E1 were confirmed in experiments with river water samples, in which E2 was converted into E1 and mineralised according first order kinetics (Equation 6) (Jürgens et al. 2002).

$$C_{E,t} = C_{E,0}\, e^{-k_d t} \tag{7}$$

In which $C_{E,0}$ is the initial concentration (ng/l) and $C_{E,t}$ is the concentration at time t (days) and k_d the reaction constant (day^{-1}). The halflife $t_{1/2}$ (days) can be calculated according Equation 8.

$$t_{1/2} = \frac{\ln 2}{k_d} \tag{8}$$

E1 can be mineralised by cleavage initiating the A-ring (Layton et al. 2000) or initiated at C-17 of ring D (Lee & Liu 2002) . In the first case, the ring is cleaved by hydroxylation at C-4, after that a oxidative fission between C-4 and C-5 by a dioxygenase, from there on it can be converted into either pyridine carbolic acid, where no CO_2 is formed, or to compound I and II from Figure 5, where CO_2 is formed (Coombe et al. 1966). However, D-ring cleavage is more likely since lactone has been identified as a metabolite, a suggestion for the pathway is given as Figure 6 (Lee & Liu 2002). Eventually, estrogens will be mineralised, as after 25 days, 24–45% of radio labelled ^{14}C E2 has been converted to CO_2 by micro-organisms from river water (Jürgens et al. 2002) and 70–80% was converted into CO_2 by

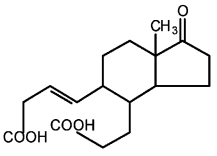
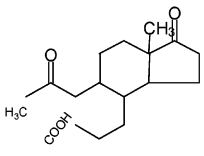
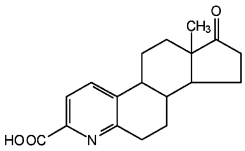
Name	I: 3aα-H-4α-[3'-propanoic acid]-5β-[2-ketopropyl]-7aβ-methyl-1-1indanone	II: 3aα-H-4α-[3'-propanoic acid]-5β-[4'-but-3-enoic acid]-7aβ-methyl-1-1indanone	pyridine carboxylic acid: 2-carboxy-7aβ-methyl-7-keto-9aαH-indano[5;4f]-5aα;10;10aβ;11-tetrahydrohydroquinoline
Structure			

Figure 5. Products after ring cleavage of estrone (E1) (Coombe et al. 1966).

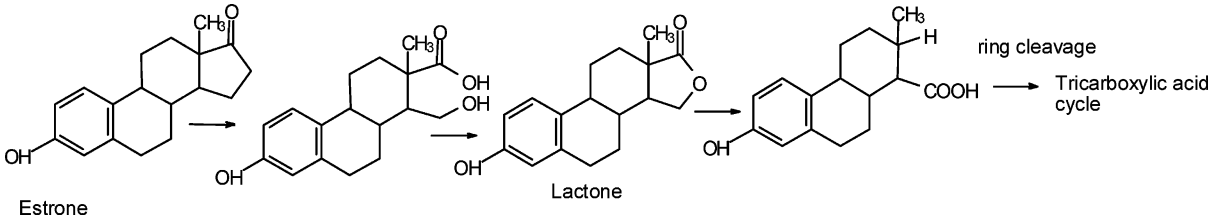


Figure 6. Suggested route of ring cleavage of estrone (E1) (Lee and Liu 2002).

sludge from municipal STPs after 24 h (Layton et al. 2000). Also EE2 can be mineralised as after 24 h 40% of ^{14}C-EE2 was converted into CO_2 (Layton et al. 2000).

All the *k*-values obtained from literature are summarised in Table 3. In this table, an attempt has been made to standardize *k*-values for the applied dry matter content in different batch tests and therefore expressed in l/gSS/day. Accordingly, the half life is calculated for 1 g SS. The general trend in the conversion rates is that the conversion of E2 to E1 is rapid, in some cases even a few minutes, and that EE2 is sometimes not converted at all, or in a far slower rate, with halflifes of 6 h up to 5 days. Natural estrogens are thought to be biodegraded via a pathway where bacteria can use the conversion for growth, as EE2 is thought to be biodegraded by co-metabolism, in which an organic compound is modified but not utilised for growth (Vader et al. 2000). Nitrifying sludge is held responsible for the conversion of EE2 by the use of the enzyme ammonium monooxygenase, which insert oxygen into C–H bonds (Vader et al. 2000). The nitrifying activated sludge converted EE2 to more hydrophilic metabolites almost completely in about six days, while sludge with a very low nitrifying capacity did not convert EE2 (Vader et al. 2000).

Using *N*-Allylthiourea (ATU), a chemical that inhibits the nitrification by blocking the ammonium monooxygenase enzyme, resulted in slower conversion of EE2, while the conversion rates of E1 and E2 remained the same (Table 3). If ATU is applied on a pure culture of nitrifying bacteria the conversion is completely blocked, as in activated sludge it was only slowed down, suggesting that in activated sludge also other bacteria are able to convert EE2.

Another remarkable trend showed in Table 3 is that the conversion appears to be a lot faster when the initial concentration of the estrogens is lower. When plotting the initial concentration against standardized *k*-values there seems to be an adverse correlation, which is even more clear when plotting the applied amount of estrogens per gram SS (charts not published). This can either indicate an inhibition of the estrogens on the sludge or it can be due to another unknown phenomenon. Inhibition by EE2 has been confirmed in a biological oxygen demand (BOD) test with activated sludge at 28 °C in the dark,

and with addition of 60 mg/l E2 or EE2. E2 is biodegraded, but addition of EE2 led to a lower BOD than the blank (Kozak et al. 2001). The latter was confirmed in a toxicity test with nitrifying sludge, a sensitive group of micro-organism towards toxicants, which shows toxic effects for concentrations above 10 mg EE2/l (Kozak et al. 2001). This trend of higher conversion rates at lower concentrations has also been found in a river water sample, when the conversion rate for E2 was slightly higher spiking with 0.1 μg/l compared with 100 μg/l, while oxygen depletion was not the case (Jürgens et al. 2002). Also Ternes et al. (1999a) observed faster degradation at 1 μg/l of E2 compared to 1 mg/l. Another example was found by Shi et al. (2004), finding faster degradation at 0.2 μg/l compared to 0.2 mg/l.

The degradation rate is depending on the temperature, as in a temperature range from 5 to 10 °C, the k_d value is 4.2 day^{-1} for E2 and 0.14 day^{-1} for EE2, while in the range of 20–25 °C, the k_d values are 6.0 day^{-1} and 0.29 day^{-1} (Jürgens et al. 2002).

Adaptation of the microorganisms is of importance as sludge from a STP was able to remove 84% of ^{14}C-E2 and 85% of ^{14}C-E1, against less than 4% by industrial sludge unexposed to estrogens (Layton et al. 2000). The industrial sludge might consist of a different bacteria population that is not capable of converting E1 and E2. The SRT of the industrial sludge is quite long (17 days) and not reported for municipal sludge, but both glucose conversion rates were the same, indicating similar biological activity. Mineralisation by STP sludge of ^{14}C-EE2 was 25–75-fold less; only 40% was converted in 24 h (Layton et al. 2000). It is not clear whether this can be explained by the presence or absence of nitrifying bacteria, or that other bacteria are capable of the conversion of estrogens. The type of sludge can also be important as shown in tests with both activated sludge and sludge from a membrane bioreactor (MBR) (Joss et al. 2004) (Table 3). MBR sludge showed a 2–3-fold faster conversion, which was explained by the longer sludge retention time of MBR sludge as the smaller floc size of MBR sludge resulting in a higher specific surface area, enhancing transfer in the floc. The SRT seems to be of most importance as shown in research comparing the degradation of EE2 in a conventional system with a very high SRT of 52–237 days, with a MBR system, no significant differences in removal were found (Clara et al. 2004a).

There have been a few attempts in order to isolate a micro-organism that can specifically convert estrogens. The fungus *Fusarium proliferatum*, has been isolated from a cowshed sample and is capable of converting EE2 (Shi et al. 2002). The fungus was able to remove 97% of EE2 at an initial concentration of 25 mg/l in 15 days at 30 °C and gave a k_d value of 0.6 day^{-1} at an optimum pH of 7.2 (Shi et al. 2002). This resembles a half-life of 1.2 day, which is remarkably faster than measured in activated sludge. The role that fungi can play is degradation by production of enzymes, as was shown in a test with direct addition of the enzyme laccase to a solution of E1 and EE2. In three days around 40% of E1 and 75% of EE2 disappeared (Tanaka et al. 2000). Fungi might also be responsible for the conversion of EE2 in STPs, since they can also be present in sludge. From an activated sludge plant, a gram-negative bacterium, possibly from the genus *Novosphingobium*, was isolated and was capable of degrading E2 and E1, but not EE2 (Fujii et al. 2002). The culture was able to degrade 60% of E2 in 14 days and 40% of E1 in 20 days. The degradation of E2 was not enhanced by the addition of yeast extract or glucose. Among 20 white-rot fungal strains have been screened for the removal capacity of a variety of (xeno) estrogens, including E1 and E2 (Fujita et al. 2002). Removal was not established in seven of the tested strains for either E1 or E2 or both, in other strains the removal varied from 5.5% to over 99.9%.

5.3. *Anaerobic biodegradation*

Little research has been completed on the fate of estrogens under anaerobic conditions, and the available data are presented in Table 4. Bed sediment was used to examine the potential for E2 to be degraded anaerobically at 20 °C; and was fairly rapidly converted to E1, almost completely after an incubation of 2-days (Jürgens et al. 2002). In batch experiments with activated sludge supernatant under anaerobic conditions (purged with nitrogen gas), after 7 days 50% of the spiked amount of E2 was converted into E1 (Lee & Liu 2002). No further degradation of E1 was observed, so E1 may accumulate as a

Table 3. First order degradation constants for bact test under aerobic circumstances for E1, E2 and EE2 (SD in brackets)

References	Sludge	Feeding conditions	Temp (°C)	Compound	Initial conc.	*k*-value (1/h)	SS (g/l)	*K* value (l/g SS/day)	$t_{1/2}$ at DS of 1 g/l	Unit
Kjoholt et al. (2004)	Activated sludge	Artificial wastewater 100 mg BOD/l at begin than 25 mg BOD/day	16	E1	500 (ng/l)	8.44	0.5	405 (158)	2.46	Minutes
				E2		16.0		768 (419)	1.3	Minutes
				E1 + E2		5.26		271 (183)	3.68	Minutes
				EE2		0.063		3.03 (1.21)	5.55	Hours
				E1-3Glu		2.58		60 (65)	8.05	Minutes
				E1-3Sulf		n.a.		0	Not degraded	
Shi et al. (2004a)	Activated sludge	Mineral salts medium	30	E1	1.0 mg/l	0.056 *0.047*	2.7	0.50 *0.42*	1.39 *1.66*	Days
				E2		1.3 *0.94*		11.56 *8.36*	1.44 *1.99*	Hours
				EE2		0.035 *0.022*		0.31 *0.20*	2.23 *3.54*	Days
				E1	0.2 mg/l	0.036 *0.035*	0.7	1.23 *1.20*	13.48 *13.86*	Hours
				E2		0.6 *0.34*		20.57 *11.66*	48.52 *85.62*	Minutes
				EE2		0.059 *0.039*		2.02 *1.34*	8.22 *12.44*	Hours
	Activated sludge + ATU			E1		0.004 *0.033*		0.14 *1.13*	5.05 *0.61*	Days
				E2		0.32 *0.34*		10.97 *11.66*	1.52 *1.43*	Hours
				EE2		0.0085 *0.008*		0.29 *0.26*	2.38 *2.63*	Hours
	Pure culture *Nitrosomas europea*			E1	0.4 mg/l	0.0084	n.a.			
				E2		0.0091				
				EE2		0.0078				
	As above + ATU			E2		0				
Vader et al. (2000)	Activated sludge,nitrifying	Hydrazine (10 mg/l)	20	EE2	50 μg/l	0.025	1	0.60	1.16	Days
	Activated sludge, low nitrifying					0		0	Not degraded	
Joss et al. (2004)	Activated sludge	No feed, only fed before exp. With final effluent	16	E1	500 ng/l	2.025	0.3	162 (25)	6.16	Minutes
				E2		4.375		350 (42)	2.85	Minutes
				EE2	100 ng/l	0.1		8 (2)	2.08	Hours
	MBR sludge			E1	500 ng/l	5.1958	0.29	430 (55)	2.32	Minutes
				E2		11.4792		950 (120)	1.05	Minutes
				EE2	100 ng/l	0.0725		6 (1)	2.77	Hours
Shi et al. (2004b)	Activated sludge	Mineral salts medium	30	E1	20–25 mg/l	0.013	2	0.16	4.47	Days
				E2		0.0183		0.22	3.15	Days
				EE2		0		0	Not degraded	
	Night soil composting sludge			E1		0.0083		0.10	6.93	Days
				E2		0.0146		0.18	3.96	Days
				EE2		0		0	Not degraded	

Ternes et al. (1999a)	Activated sludge	No feed	n.a.	E1	1 mg/l	0.042	0.26	3.88	4.29	Hours
				E2		1.08		99.69	10.2	Minutes
				EE2		0		0	Not degraded	
Ternes et al. (1999a)	Activated sludge	No feed	n.a.	E1	1 μg/l	0.48	0.52	22.15	45.05	Minutes
				E2		10.3		475.38	2.10	Minutes
				EE2		0		0	Not degraded	
Kikuta and Urase (2003)	Activated sludge, at pH 6.7	Artificial wastewater	20	E1	100 μg/l	0.109	2.663	0.98	16.93	Hours
				E2		6.84		61.64	16.19	Minutes
				EE2		0.013		0.12	5.92	Days
	Activated sludge, at pH 5.6			E1		0.263	2.774	2.28	7.31	Hours
				E2		8.39		72.59	13.75	Minutes
				EE2		0.105		0.91	18.31	Hours
	Activated sludge, at pH 4.4			E1		0.167	2.189	1.83	9.09	Hours
				E2		13.33		146.14	6.83	Minutes
				EE2		0.088		0.96	17.24	Hours
Fujii et al. (2002)	Gram negative bacterium isolated from activated sludge	Mineral salts medium	25	E2	1 g/l	0.0016	0.8E + 3 tot	n.a.		
					0.3 g/l	0.0024	0.5E + 09			
					0.17 g/l	0.0033	CFU/ml			
Onda et al. (2003)	Activated sludge	No feed	20	E1	16 μg/l	4.19[a]	2	50.28	19.85	Minutes
				E2		5.85[a]	2	70.32	14.19	Minutes
Layton et al. (2000)	Activated sludge	No feed	5–10	E2	58 μg/l	0.174	2.165	1.93	8.62	Hours
				EE2	72 μg/l	0.006		0.07	10.42	Days
			22–25	E2	58 μg/l	0.252		2.79	5.96	Hours
				EE2	72 μg/l	0.012		0.13	5.21	Days
Lee and Liu (2002)	E2 degrading bacteria	Mineral salts medium	21	E2	200 μg/l	0.048[b]	n.a.		14	Hours
						0.021[c]			1.4	Days
Shi et al.(2002)	Fungus *F. proliferatum*	No feed	30	EE2	25 mg/l	0.025			1.2	Days

SS = suspended solids, ATU = chemical that inhibits nitrification, n.a. = not available.
When no values were available least-square method was used for calculation. For Shi et al. (2004) *k*-values have been recalculated, since the given values in the article did not give an accurate curve-fit recalculated figures in italic.
[a]Estrogens only measured in the supernatant; therefore, the *k*-value is an overestimation since it also includes adsorption.
[b]Cyclone fermentor.
[c]Rotary shaker.

by-product. Autoclaved samples were used as sterile controls. EE2 tested under anaerobic conditions in river water samples showed no degradation over 46 days (Jürgens et al. 1999). Under strict anaerobic conditions E1 is expected to convert into E2, rather than E2 is converted to E1. This pathway was shown by Joss et al. (2004), who found a standardized *k*-value of approximately 20 min and also a conversion of E2, with a *k*-value of 6 min in activated sludge and 2 min in MBR sludge. So somehow under anaerobic conditions there are still electron acceptors available, like Fe^{3+} and various organic oxidative compounds, responsible for the conversion. Joss et al. (2004) even found conversion of EE2 in MBR sludge under anaerobic conditions of about 1.5 l/g/day, but this value is nearly the same as the degradation figure derived from the blank experiment, where no sludge was present. Overall anaerobic conditions resulted in much slower conversion rates compared to the same experiments under aerobic conditions. For example a half life of 2.5 min for E1 under aerobic conditions, was 1.66 h under anaerobic conditions (Table 3 and 4). No degradation of the three estrogens was found by (Pakert et al. 2003) in batch tests with sludge from and anaerobic sludge digester.

5.4. *Anoxic biodegradation*

First order conversion rates under anoxic conditions are shown in Table 5. Under anoxic conditions the conversion rates lay in between those under anaerobic and aerobic conditions. For example the degradation for EE2 was 11 h under anaerobic conditions, 2.8 h under aerobic and 5.6 h under anoxic conditions (Joss et al. 2004) (Tables 3– 5).

5.5. *Photolytic degradation*

Photolytic degradation of E2 and EE2 occurs; approximately 40% of the initial concentration was left after 144 h in a spectral distribution similar to natural sunlight, while no degradation in the dark controls was observed (Layton et al. 2000). The half life is 124 h for E2 and 126 h for EE2, so it would take at least ten days to degrade the components to half the initial concentration and is therefore slow compared to the biodegradation of E2. For EE2 it might be more significant, since the halflife for biodegradation is 17 days in rivers (Layton et al. 2000). Experiments by Segmuller et al. (2000) to identify autooxidation and photodegradation products of EE2 shows a series of isomeric dimeric oxidation products, a molecule that exists of two EE2 molecules. This molecule might have lost estrogenic properties, but no information was provided on its stability in the environment.

6. Pilot plant and full scale STPs

The efficiency of a STP to degrade estrogens is influenced by numerous parameters including microbial activity, sludge retention time (SRT),

Table 4. First order degradation constants for batch test under anaerobic circumstances for E1, E2 and EE2

References	Sludge	Feeding conditions	Temp (°C)	Compound	Initial conc.	*k*-value (1/h)	SS (g/l)	*K*-value (l/g SS/day)	$t_{1/2}$ at SS of 1 g/l	Unit
Joss et al. (2004)	Activated sludge	No feed, only fed before exp. With final effluent	16	E1	500 ng/l	0.1250	0.3	10 (1)	1.66	Hours
				E2		2.1875		175 (10)	5.70	Minutes
				E1 red. to E2		0.6500		52 (2)	19.19	Minutes
	MBR sludge			E1		0.2567	0.22	28 (3)	35.65	Minutes
				E2		4.5833		500 (200)	2.00	Minutes
				EE2	100 ng/l	0.0138		1.5 (0.5)	11.09	Hours
				E1 red. to E2	500 ng/l	0.5500		60 (15)	16.64	Minutes
Lee and Liu (2002)	E2 degrading bacteria	Mineral salts medium	21	E2	200 μg/l	0.0024	n.a.		12	Days

red = reduction, SS = suspended solids (SD in brackets).

Table 5. First order degradation constants for batch test under anoxic circumstances for E1, E2 and EE2

References	Sludge	Feeding conditions	Temp (°C)	Compound	Initial conc.	*k*-value (1/h)	SS (g/l)	*K*-value (l/g SS/day)	$t_{1/2}$ at DS of 1 g/l	Unit
Kjoholt et al. (2004)	Activated sludge	Artificial wastewater 100 mg BOD/l at begin than 25 mg BOD/day	16	E1	500 ng/l	0.2900	0.5	14 (5)	1.20	Hours
				E2		10.3500		497 (283)	2.01	Minutes
				E1 + E2		0.5600		27 (21)	37.5	Minutes
				EE2		0.0035		0.17 (0.17)	4.13	Days
Joss et al. (2004)	Activated sludge	No feed, only fed before exp. With final effluent	16	E1	500 ng/l	0.3750	0.3	30 (10)	33.27	Minutes
				E2		5.75		460 (60)	2.17	Minutes
				EE2	100 ng/l	0.0150		1.2 (0.3)	13.86	Hours
	MBR sludge			E1	500 ng/l	1.2938	0.27	115 (30)	8.68	Minutes
				E2		3.1500		280 (50)	3.56	Minutes
				EE2	100 ng/l	0.0338		3 (2)	5.55	Hours

SS = suspended solids (SD in brackets).

hydraulic retention time (HRT), temperature, and rainfall, all of which vary seasonally (Ternes 1998). Different treatment systems may also affect microbial activity and therefore, estrogenic composition of STP effluents (Rodgers-Gray et al. 2000). Conventional sewage treatment is typically a three-stage process (Metcalf & Eddy 2003). Consisting of preliminary treatment, including removal of coarse matter and grit, primary sedimentation and secondary treatment, including biological treatment like activated sludge tanks, nitrification and denitrification tanks, followed by a secondary clarifier and sometimes tertiary treatment. Sludge is dealt with during separate sludge treatment.

Before entering a STP, besides deconjugation, (anaerobic) biodegradation and sorption to particulates takes place in the sewers (Johnson et al. 2000), which can be significant in large catchment areas (Langford & Lester 2003). It is not always clear if measured influent and effluent samples were determined on total estrogens including the conjugated, but in most cases only the unconjugated were determined as it was assumed most of the de-conjugation has already taken place in the sewers. The detection methods used are in most cases only suitable to detect unconjugated hormones.

During primary sedimentation, the removal of hormones is mainly due to adsorption onto sludge. Secondary treatment can consist of an anaerobic pre-treatment but is usually an activated sludge system. In biological systems removal is due to adsorption, biological and chemical degradation. A long HRT allows more time for adsorption and degradation, while a longer SRT has influence on the biota and physical nature of floc particles, improving the sorption capacity and biodegradation (Johnson et al. 2000). At short SRT (< 8 days), slow growing specific degraders can be washed out and adsorption will be the main process for removal (Jacobsen et al. 1993).

Measurements in full scale STPs are shown in Table 6, showing all individual values for E1, E2 and EE2 and Table 7 shows values for the total estrogenity. Table 6 also shows the removal percentages. Many measurements have been conducted in the effluents of STPs, but only when information is available on both influent and effluent, the values are reported. An overall chart shows average values for influent and effluent including the standard deviation, amongst all measurements taken on full-scale plants so far and is presented as Figure 7.

The levels of estrogens found in STP effluents range from below the detection limit to 100 ng/l (Desbrow et al. 1998). STPs have the ability to remove natural steroid hormones from influents over a range of temperatures but may be less effective in removing the synthetic EE2 (Layton et al. 2000). This is illustrated by Figure 7, showing an average removal in STPs of 60% for E1, 78% for E2 and 49% for EE2 measured in STPs. Removal of E1 shows a great variation, while E2 is often removed to a level below detection limits. Removal of E1 varies from 10% as measured in Germany (Ternes et al. 1999b) to 98% as

Table 6. Actual concentrations of E1, E2 and EE2, measured in influents and effluents of STPs

References	Country	Location	Method of treatment	Detection method	Estrone E1 (ng/l)			17β-Estradiol E2 (ng/l)			17α-Ethynylestradiol EE2 (ng/l)		
					Influent	Effluent	% removal	Influent	Effluent	% removal	Influent	Effluent	% removal
Baronti et al. (2000)	Italy	Cobis	Activated sludge	LC-ESI-MS-MS	71 (35)	9.62 (5)	86 (10)	16.1 (7)	1.48 (1.02)	91(8)	3.93 (5.14)	0.64 (0.31)	84 (23)
		Fregene			67 (18)	4.06 (1.5)	94 (3)	9.2 (5)	0.92 (0.74)	90(10)	3.39 (2.35)	0.68 (0.68)	80 (24)
		Ostia			50.6 (13)	44.62 (25)	12 (54)	14.68 (7)	2.44 (1.19)	83(11)	2.48 (1.76)	0.79 (0.32)	68 (26)
		Roma Sud			35.2 (10)	30.34 (16)	14 (52)	8.6 (2.3)	1.89 (0.94)	78(12)	2.95 (2.08)	0.66 (0.37)	78 (20)
		Roma Est			50.4 (14)	7.66 (2.6)	85 (7)	9.3 (2)	0.75 (0.08)	92(2)	2.28 (1.57)	0.44 (0.20)	81 (16)
		Roma Nord			36.8 (8)	13.88 (15)	62 (41)	11.46 (3)	0.98 (0.55)	91 (5)	2.95 (2.33)	0.48 (0.10)	84 (13)
Belfroid et al. (1999a)	Netherlands	STP A'dam-Westpoort (Oct)	Aeration tank	GC/MS/MS *Italic:after addition of enzyme for unconjugation*	87; *200*	2.1;*2.1*	99	9.5; *10*	<0.6; <0.6	94	1.3; *1.5*	<0.3; 0.5	67
		STP A'dam-Westpoort (Dec)			140	47	66	48	12	75	9.7	7.5	23
		STP Kralings veer (Dec)	Carousel		100	6.3	94	31	0.7	98	8.8	<0.2	98
		STP Eindhoven (Oct)	Aeration tank		*10.3* (1)	2.7; *5.4*	48	*11*	n.a.		*9.2*	<1.4; <1.4	84
		STP Eindhoven (Dec)			42	15	64	14	1.1	92	<0.2		
Vethaak et al. (2002)		Several plants	Activated sludge	GC/MS/MS	20–130	<0.3–11		17–150	<0.8	70	<0.3–5.9	<0.3–2.6	
Bruchet et al. (2002)	France	STP Esniere sur Oise	Activated sludge	GC/MS	20	8	60	10	3		2.4	1.4	42

Cargouët et al. (2004)		Evry	Activated sludge + (de) nitrification	GC/MS	17.6 (0.5)	7.2 (0.8)	59 (5)	11.1 (1.7)	4.5 (1.4)	59 (14)	5.4 (0.6)	3.1 (0.6)	43 (13)
		Valenton			15.2 (1.8)	6.5 (1.2)	57 (9)	17.4 (1.7)	7.2 (0.8)	59 (6)	7.1 (0.9)	4.4 (1.2)	38 (19)
		Colombes	Upflow Biolfilters		9.6 (1.5)	4.3 (0.6)	55 (9)	11.6 (0.6)	6.6 (1.4)	43 (12)	4.9 (1.0)	2.7 (0.8)	45 (20)
		Achères	Activated sludge		11.2 (2.3)	6.2 (0.8)	45 (13)	17.1 (0.6)	8.6 (0.9)	50 (6)	6.8 (1.4)	4.5 (0.8)	34 (18)
Lee and Peart (1998)	Canada	Burlington (Dec)	Activated sludge	GC/MS	26 (1)	6 (0.5)	77 (2)	7 (0.6)	<5	>29 (6)	n.a.		
		Burlington (Jan)			53 (4)	8 (1)	85 (2)	14 (2)		>64 (5)			
		Dundas			69 (1)	9 (1)	87 (1)	7 (0.7)		>29 (7)			
		Edmonton			109 (5)	72 (2)	34 (4)	<5		n.a.			
		Guelph (Dec)			66.5 (12)	17 (1)	74 (5)	<5					
		Guelph (Jan)			41 (4)	14 (1)	66	15 (2)		67 (4)			
Nasu et al. (2001)	Japan	27 plants autumn	Activated sludge	ELISA	n.a.			0.03–0.090 0.042med.	n.d.–0.043 0.013 med.	69			
		Winter						0.02–0.094 0.047 med.	0.0003–0.03 0.014 med.	70			
Lebietzka (1996), p. 257, quoted in Jürgens et al. (1999)	Germany	Zittau STW	Activated sludge	EE2 ELISA	n.a.						6.8	8.0	−18
Petrovic et al. (2002)	Spain	Calaf	n.a.	HPLC-LCMS	<2.5–115	<2.5–8.1	n.a.	11.0–30.4	<5.0	n.a.	<5.0		n.a.
		Igualada			<2.5–4.6	<2.5–2.7		<5.0	<5.0–7.6				
		Piera			<2.5–13.1	<2.5		<5.0	<5.0				
		Manresa			<2.5–56.5	<2.5–7.2		<5.0–14.5	<5.0				
Shore et al. (1993)	Israel	Tel Aviv	(1) anaerobic tank	E2 Radio-immunoassay	48–141	7–39	22–64	n.a.					
			(2) activated sludge		7–39	6–50							

Table 6. Continued

References	Country	Location	Method of treatment	Detection method	Estrone E1 (ng/l)			17β-Estradiol E2 (ng/l)			17α-Ethynylestradiol EE2 (ng/l)		
					Influent	Effluent	% removal	Influent	Effluent	% removal	Influent	Effluent	% removal
Tabak et al. (1981)	USA	Batavia	Trickling Filter	TLC and GLC after hydrolysis and liquid/ liquid extraction	20 (20)	10 (7)	50 (61)	10	<10		1070 (810)	670 (590)	37 (73)
		Fairfield	Activated sludge		10 (7)	10	0 (70)	10	<10		890 (630)	530 (430)	40 (64)
		Lebanon			20 (14)	10	50 (35)	10	<10		880 (520)	540 (370)	39 (56)
		Milford	Trickling filter		40 (20)	20 (14)	50 (43)	10 (7)	10		1330 (610)	910 (630)	32 (57)
		New Richmond	Contact stabilisation		40 (20)	20 (7)	50 (31)	20 (7)	10	50 (18)	1290 (710)	920 (580)	29 (60)
		Bethel	Trickling filter		30 (20)	20 (14)	33 (64)	10 (7)	10		1000 (640)	660 (470)	34 (63)
		Loveland			30 (20)	10 (7)	67 (32)	10 (7)	10		990 (520)	630 (400)	36 (52)
		Little Miami	Primary		50 (30)	30 (14)	40 (46)	20 (7)	10 (7)	50 (39)	1770 (710)	1320 (640)	25 (47)
		Bromely	Contact stabilization		40 (14)	10 (14)	75 (36)	10 (7)	10		1480 (640)	1040 (530)	30 (47)
		Muddy Creek	Primary		50 (20)	30 (14)	40 (37)	20 (7)	10	50 (18)	1590 (750)	1160 (660)	27 (54)
		Hamilton	Activated sludge		20 (14)	10 (7)	50 (50)	10	<10		1270 (400)	780 (320)	39 (32)
		Glendale	Trickling Filter		30 (20)	20 (7)	33 (50)	10 (7)	10		1000 (690)	600 (490)	40 (64)
Adler (2001)	Germany	Southern and middle Germany	Five STPs	GCMS, Unconjugated	<0.5–20, 2 med.	<0.1–18, 0.3 med.	87	<0.5–4, 2 med.	<0.05–0.6, 0.2 med.	70	1–14, 12 med.	<0.05–0.6,0.2 med.	98
				After adding enzymes	2–16, 3 med.	<0.1–57, 0.4 med.		1–22, 3 med.	0.2–2, 0.9 med.		1–45, 26 med.	<0.1–2, 0.4 med.	

			Seven STPs	GCMS, Unconjugated	2–25, 6 med.	<0.05–130, 3 med.	14	1–9, 1 med.	<0.05–4, 0.7 med.	30	1–9 4 med.	<0.1–4, 0.5 med.	71
				After adding enzymes	8–25, 14 med.	<0.1–170, 12 med.		2–19, 3 med.	0.2–6, 3 med.		5–15, 7 med.	<0.1–40, 2 med.	
Andersen et al. (2003)	Germany	Wiesbaden	(1) primary clarifier	GCMS	65.7	74.9	−14	15.8	10.9	31	8.2	5.2	37
			(2) denitrification-1		74.9	37.3	50	10.9	10.3	6	5.2	1.5	71
			(3) denitrification-2		37.3	2.8	92	10.3	<1	90	1.5	1.2	20
			(4) nitrification		2.8	<1	64	<1	1.8	−80	1.2	<1	>20
			(5) secondary clarifier		<1	<1		1.8	<1		<1	<1	
			Total removal				>98			>94			>88
			(2) denitrification-1	GCMS; on sludge(ng/g)	10.1			2.7			1.9		
			(3) denitrification-2		6.9			2.3			2.2		
			(4) nitrification		5.6			2.2			<1.5		
			(6) digested sludge (solid)		25.2			5.1			<1.5		
			(6) digested sludge(liquid)		67.1 (ng/l)			5.4 (ng/l)			<1 (ng/l)		
Schullerer et al. (2002)		Karlsruhe	(1) activated sludge with N and P removal	GC/MS	130	26	80	32	3.2	90	55	9.0	84
			(2) trickling filter		26	11	58	3.2	2.7	16	9.0	7.0	22
							92			92			87
		Ebingen	(1) primary clarifier		120	65	46	35	17	51	20	n.d.	>91
			(2) activated sludge with N and P removal		65	2.1	97	17	6.0	65	n.d.	2.7	
			(3) activated carbon		2.1	n.d.		6.0	n.d.		2.7	1.8	33
							>99			>97			91
		Lautlingen	(1) primary clarifier		49	34	31	31	29	6	59	55	7
			(2) activated sludge with N and P removal		34	3.3	90	29	1.3	96	55	3.7	93
			(3) activated carbon		3.3	n.d.		1.3	n.d.		3.7	2.2	41
							>98			>97			96

med. = median value, n.a. = not available.
SD in brackets if available.
Standard deviations for the removal are calculated according propagation of uncertainty (Rubinson 2000).

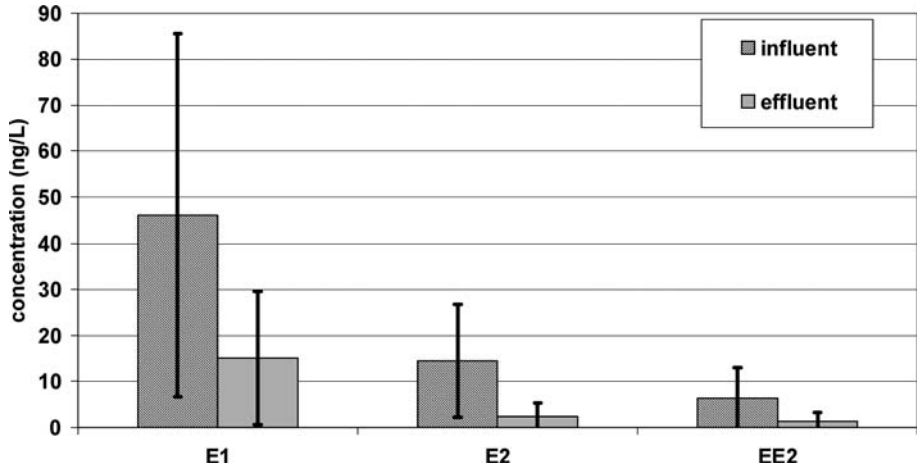


Figure 7. Average concentrations in influent and effluent over all researched STPs included error bars for the standard deviations, based on Table 6.

measured in the Netherlands (Belfroid et al. 1999a) and also tends to vary within the same STP. The type of treatment as well as the process conditions of each particular treatment may cause the large variance in removal. The large standard deviation for E1 in effluents of some STPs might indicate that mineralisation is not established every time.

From Table 6, removal percentages for estrogens are calculated for the different processes in the treatment chain. The major reduction occurs in almost all cases during biological secondary treatment. The actual removal rates will be a reflection of the ability of the indigenous micro-organisms to biodegrade the compounds within a hydraulic residence time of approximately 14 h, and also of the ability of the floc particles to bind the compounds (Johnson et al. 2000).

The fate of estrogens along the treatment chain, as shown as Figure 8, will be discussed below. All values discussed can be found in Tables 6 and 7.

6.1. *Primary clarifier*

The initial estrogenity sometimes rises after the primary clarifier, which may indicate that deconjugation is not always complete when the wastewater enters the STPs (Kirk et al. 2002). In the German plant located at Wiesbaden, E1, E2 and EE2 are individually analysed and show a negative removal for E1 and positive for both E2 and EE2, presumably due to conversion of E2 into E1 and the better sorption capacities of EE2. It is highly unlikely that the latter is degraded under the anaerobic conditions in a clarifier. Addition of precipitates during primary treatment such as aluminium or iron does not increase the removal, with the exception of lime with which a removal of 73% was accomplished. The high pH of 11.4, which is above the isoelectric point, means that the estrogens are present in the dissociated form, may have an influence, as the pH at addition of the other precipitates was between 6.3 and 7.3 (Svenson et al. 2003). Clara et al. (2004b) found that using milk of lime in batch adsorption experiments, less E2 and EE2 was sorbed due to increased solubility, so from this point of view, no increase in removal is expected using lime. However, for a pH above 12 there seems to be a drop in concentration for EE2, so at this point some form of coagulation for this particular compound could occur.

6.2. *Anaerobic stage*

The reaction fluid of an anaerobic tank in Tel Aviv, Israel was analysed for E2. The removal was 72–85%, which is in line with the expecta-

Table 7. Influent, effluent concentrations of total estrogenity

References	Country	Location	Treatment	Compound detection	Influent (ng/l)	Effluent (ng/l)	HRT/SRT	% removal per stage	% tot. removal of whole plant
Svenson et al. (2003)	Sweden	Bureå	Precipitation; Al	Tot. estr. In equivalent E2	11.90 (1.27)	12.40 (1.41)	NR	−4%	
		Järpen	Precipitation; Al	YES	10.80 (0.14)	12.70 (1.70)		−18%	
		Sörsjön	Precipitation; Fe(III)		5.45 (0.21)	5.9 (0.30)		−8%	
		Skulsjön	Precipitation; lime		4.15 (0.78)	1.1		73%	
		Enköping	Activated sludge		29.8	12.3 (0.28)	2–8 h/NR	59%	
		Kattastrand	Activated sludge		5.00 (0.57)	0.3 (0.28)		94%	
		Sollebrunn	Activated sludge		10.20 (0.14)	4.3 (0.14)		58%	
		Reffelmansverket	Activated sludge		5.00 (0.71)	1.6 (0.28)		68%	
		Ljusne	Activated sludge		6.05 (0.21)	1.2		80%	
		Nolhaga	Trickling filter		22.35 (2.47)	14.85 (2.19)		34%	
		Gårlången	Trickling filter		3.05 (0.21)	10.75 (0.92)		−252%	
		Hassela	Biorotor		1.60 (0.71)	5.25 (0.21)		−228%	
		Sundet	Activated sludge		8.00 (0.42)	2.55 (0.35)		68%	
		Uddebo	Trickling filter		6.75 (0.21)	1.7		75%	
		Vimmerby	Tricklingfilter/ activated sludge		6.95 (1.2)	0.1	12 h/NR	99%	
		Kungsängsverket	Activated sludge		12.50 (0.57)	1.45	NR	88%	
		Kävlinge	Activated sludge + nitrogen removal		3.85 (1.48)	<0.1	20 h/NR	>74%	
		Ekebyverket	Activated sludge + wetland		19.5 (2.26)	<0.1	7 d/NR	>99%	
Takigami et al. (2000)	Japan	Shiga night soil plant	(1) Influent (mix nightsoil septage)	Tot. estr. In equivalent E2	4000[1]–650[2]		15 day/NR		>99.9%; 99.9%
			(2) denitrifiation-1	YES[1] and ELISA for E2[2] in the liquid phase		202[1]–43.8[2]			
			(3) nitrification		202[1]–43.8[2]	123[1]–29.3[2]		97%; 96%	
			(4) denitrifiation-2		123[1]–29.3[2]	43.4[1]–18.1[2]		39%; 33%	
			(5) sedimentation tank		43.4[1]–18.1[2]	6.9[1]–11.1[2]		65%; 38%	
			(6) flocculation		6.9[1]–11.1[2]	7.47[1]–8.88[2]		84%; 39%	
			(7) ozonation		7.47[1]–8.88[2]	2.2[1]–1.41[2]		−8%; 20%	
			(8) sand filtration		2.2[1]–1.41[2]	n.d.[1]–0.824[2]		71%; 84%	
			(9) activated carbon		n.d.[1]–0.824[2]	n.d.[1]–0.124		–; 41%	
			(10) dewatering filtrate		5.11[1]–4.23[2]			–; 85%	

Table 7. Continued

References	Country	Location	Treatment	Compound detection	Influent (ng/l)	Effluent (ng/l)	HRT/SRT	% removal per stage	% tot. removal of whole plant
			(1) night soil	As above but in the sludge phase (ng/g dry matter)	624[1]–303[2]				
			(1) septic tank sludge		1120[1]–274[2]				
			(2) denitrifiation-1		305[1]–102[2]				
			(3) nitrification		159[1]–101[2]				
			(4) denitrifiation-2		274[1]–107[2]				
			(5) concentrated sludge		322[1]–101[2]				
Matsui et al. (2000)[a]	Japan	Shiga	(1) primary sedimentation	Tot. estr. In equivalent E2	150[1]–50[2]	140[1]–70[2]	NR	7%; −40%	93%; 80%
			(2) denitrification	YES[1] and ELISA for E2[2]	140[1]–70[2]	10[1]–20[2]		93%; 71	
			(3) nitrification		10[1]–20[2]	10[1]–15[2]		0%; 25%	
			(4) secondary sedimentation		10[1]–15[2]	10[1]–10[2]		0%; 33%	
			(5) chlorination/sandfilter		10[1]–10[2]	10[1]–10[2]		0%; 0%	
			(6) dewatering filtrate		470[1]–140[2]				
Bolz et al. (2000)	Germany	KA1	(1) primary clarifier	Tot. estr. In equivalent E2	13	2.4	NR	82%	99%
			(2) activated sludge with N and P removal	(E-screen)	2.4	1.5		38%	
			(3) activated carbon		1.5	0.36		76%	
			(4) secondary clarifier		0.36	0.12		67%	
			(5) final effluent		0.12	0.19		−58%	
		KA2	(1) primary clarifier		82	65		21%	99%
			(2) activated sludge with N and P removal and activated carbon		65	n.d.			
			(3) final effluent		n.d.	0.74			

	KA3	(1) primary clarifier		22	17		23%	93%
		(2) trickling filter		17	2		88%	
		(3) activated sludge nitrification and P removal		2	6.4		−120%	
		(4) final effluent		6.4	1.6		75%	
	KA4	Activated sludge with N and P		16	1.3		92%	92%
	KA5	(1) primary clarifier		38	32		16%	95%
		(2) activated sludge with N and P removal		32	1.9		94%	
	KA6	Tricklingfilter with N and P removal		46	2.6		94%	94%
Kirk et al. (2002)[a] UK	WWTW A	Primary treatment	Tot. estr. In equivalent E2	43[1]; 17[2]	40[1]; 14[2]	2–6 h/NR		7%; 18%
	WWTW B	(1) secondary treatment	YES [1]data April; May	75[1]; 22[2]	10[1]; 2[2]	4 h/NR	87%; 91%	89%; 68%
		(2) pre UV treatment	[2] data August	10[1]; 2[2]	7[1]; 3[2]		30%; −50%	
		(3) final effluent		7[1]; 3 [2]	8[1]; 7[2]		−14%; −133%	
	WWTW C	(1) primary treatment		38[1]; 10[2]	30[1]; 23[2]	13.5 h/NR	21%; −130%	>97%; 80%
		(2) secondary treatment		30[1]; 23[2]	5[1]; 2[2]		83%; 91%	
		(3) Biofilter		2[1]; 2[2]	nd[1]; 2[2]		–; 0%	
	WWTW D	(1) primary treatment		33[1]; 27[2]	13[1]; 14[2]	13 h/NR	61%; 48%	>97%; 96%
		(2) final effluent		13[1]; 14[2]	n.d.[1]; 1[2]		>99%; 93%	
	WWTW E	(1) primary treatment		77[1]; 40[2]	27[1]; 17[2]	13 h/NR	65%; 58%	>98%; 83%
		(2) slag filtration		27[1]; 17[2]	13[1]; 13[2]		52%; 24%	
		(3) plastic filtration		27[1]; 17[2]	13[1]; 12[2]		52%; 29%	
		(4) activated sludge		27[1]; 17[2]	n.d.[1]; 7[2]		>99%; 59%	

SD in brackets. NR = not reported, n.a. = not detected.
[a]Values obtained from a chart.

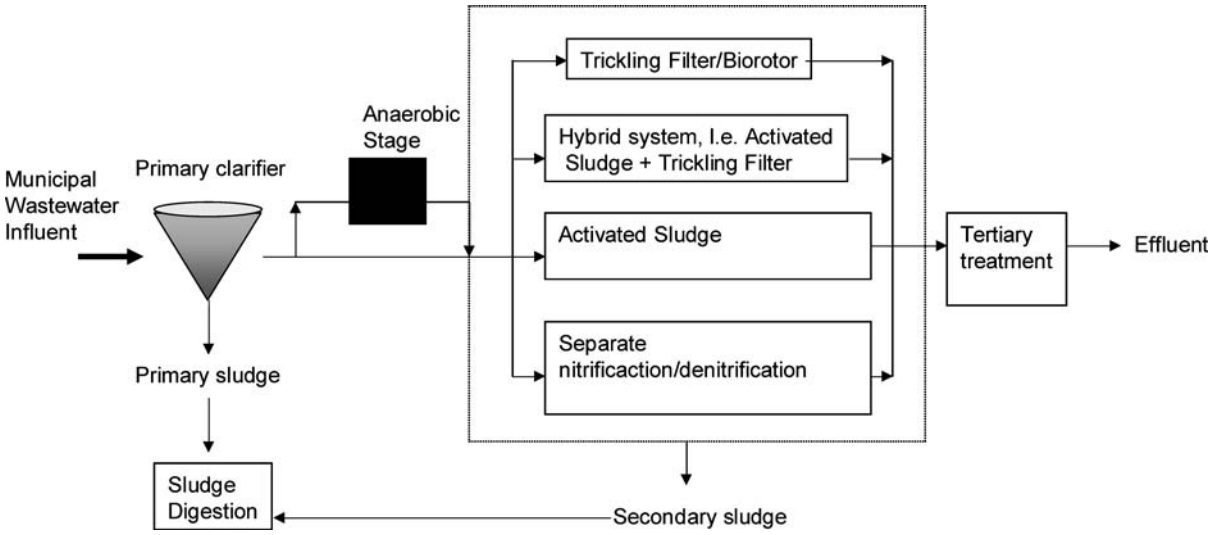


Figure 8. Schematic review of the treatment chain and the different processes that are described for estrogen removal.

tions, as E2 can be converted to E1, also under anaerobic conditions.

6.3. *Activated sludge*

Measurements done inside the aerated phase of the activated sludge system in the STP of Zittau, Germany and Tel Aviv, show a negative removal value (data not shown in Tables). It seems that all estrogens are set free, as if they de-adsorb. Often deconjugation of the sulphate-conjugates is used as explanation, but the increase is higher than expected on base of the amounts of (sulphate) conjugates present in the influent. On average, 58% of total E1, 58% of total E2 and 26% of total EE2 were conjugated in raw sewage as measured in 7 STPs in South and Central Germany. During treatment approximately 27% of E1, 67% of E2 and 92% of EE2 is split and the total amount of conjugates in the effluent remains 45% (Adler 2001). This is explained by lack of arylsulphatase, which may be plausible as E1 is mainly excreted as sulphate conjugates and most of E1 is still present in a conjugated form in the effluent (Baronti et al. 2000). Another explanation for the increased level of estrogens in the aeration tank is de-adsorption of estrogens from sludge during the analysis, since it did not become clear whether the total sample was analysed or only the liquid phase. In Zittau, Germany, the effluent of the activated sludge process contains a higher amount of EE2 than the influent, which may be a combination of deconjugation and low biodegradability of the latter compound. During the aerobic phase of activated sludge treatment, the removal is fairly good, although sometimes a concentration over 10 ng/l is still present in the effluent. Removal percentages show a high variation between different STPs. The occurrence of nitrification in an activated sludge system seems to have a positive effect on the removal of hormones. For nitrification a longer SRT is required because the autotrophic bacteria involved grow very slow.

6.4. *Trickling filter/biorotor*

Removal percentages for estrogens are quite variable in a trickling filter or biorotor as can be seen in Table 6. They are even negative, which might be due to the relatively short hydraulic retention times in such systems, which are in the range of several minutes for high rate trickling filters and 2 h for low rate tricking filters (Metcalf & Eddy 2003), so the available time for adsorption or biodegradation is short.

6.5. *Hybrid techniques*

From hybrid techniques, in which an activated sludge tank is followed up by another treatment step like a trickling filter, biorotor or wetland,

the activated sludge in combination with a wetland shows by far the best removal for estrogens (over 99%). Retention times in wetlands are in general quite long, which explains this high removal percentage. In this case the retention time was 7 days while retention times in conventional activated sludge systems is typically 14 h (Svenson et al. 2003).

6.6. *Separate nitrification/denitrification*

From Tables 6 and 7 it is shown that the highest percentage removal of estrogens from the liquid phase is obtained during the denitrification step. Even when a second denitrification step is applied, more is removed during denitrification compared to nitrification, so it is not just due to higher concentrations in the influent or solely to a dilution of primary effluent by the return sludge and internal recirculation with low estrogen concentrations. On one occasion the concentration of E2 is increasing during the nitrification step. As this is the last step in the treatment chain, it is highly unlikely that this is due to deconjugation, though it can not be excluded; as mentioned previously that still around 40% can be present in the conjugated form in the final effluent. Perhaps the bonding of E2 onto sludge is not as strong as for the other two estrogens. In this same system, the removal of E2 in the first denitrification tank, which is receiving a nitrified stream from the nitrification tank, is also quite low, 6%, while the removal of EE2 is remarkably high, 71%. In the second tank, the removal of E2 is 90%. It appears that anoxic conditions enhance the adsorption of E1, E2 and EE2, since conversion rates have found to be lower in batch test under anoxic conditions compared to aerobic conditions, as described earlier.

6.7. *Tertiary treatment*

In general secondary sedimentation does not lead to an additional removal of estrogens, except for the activated sludge system treating night soil/septic tank sludge (night soil = human physiological waste collected in the morning). This significant removal could be explained by settling of colloidal material that is formed during the treatment of this type of influents, on which estrogens are sorbed.

Other tertiary treatment systems like chlorination, sand filter, flocculation and biofilter do not contribute to additional removal of estrogens, except for systems using activated carbon or ozonation, both able to remove over 80%. UV treatment is leading to a negative removal of estrogens measured in the liquid phase. This can be explained by the release of estrogens by destruction of sludge particles under influence of UV, which sets the estrogens free.

6.8. *Mass balances*

On some occasions, measurements of estrogens have been carried out in the sludge itself. The highest concentration of total estrogens adsorbed to sludge was measured in the influent of the night soil/septic tank sludge treatment plant which was approximately 1120 ng E2 equivalents/g dry matter for septic tank sludge compared to 624 ng/g dry matter in raw night soil, the same tendency was found measuring E2 specifically with ELISA which gave 303 and 274 ng E2/ g dry matter for septic tank sludge and night soil respectively (Takigami et al. 2000). Apparently, these values are high as conjugated hormones may have been converted into unconjugated active estrogens during their previous stay in the septic tank, while the actual degradation of these compounds is absent or very low. In the activated sludge samples in the same night soil/septic tank sludge treatment system, the E2 equivalents ranged form 159–322 ng/g dry matter, with 100 ng/g due to E2. In the activated sludge tank, 20% of the total present E2 is bound to the sludge, 50% of the E1 and 60% of the EE2 (Schwarze-Scharfenberg et al. 2003).

Least estrogens are bound to sludge inside nitrification tanks, 10 ng/g dry matter, which is in line with the hypothesis that during nitrification estrogens might desorb.

Desorption is also occurring during sludge treatment, as estrogen measurements in the dewatering filtrates are rather high, 67.1 ng/l for E1, 5.4 ng/l for E2 and EE2 was under the detection limit (Andersen et al. 2003). This phenomenon is supported by (Kunst et al. 2002).

From the total amount of estrogens in the influent, only a very small proportion is discharged with the excess sludge from a treatment plant. In the plant in Wiesbaden, Germany,

the total removal of the liquid phase is over 99% for E1 and E2, while only 4% of the incoming E1 and E2 is removed with the excess sludge. For EE2, over 88% is removed form the liquid phase and 5% of the incoming EE2 is removed with excess sludge. In a pilot plant, consisting of a 2.5 m^3 activated sludge tank and a settler treating pre-settled domestic wastewater, the average removal measured over one year for E1 and E2 from the liquid phase was 70 and 95% and approximately 3% of E1 and 1% of E2 leaves the plant with sludge discharge (Onda et al. 2003). Inside the aeration tank, 85% of the total amount of E1 in the tank is sorbed to sludge and 95% for E2, assuming a completely mixed tank and the same amount of estrogens sorbed onto the sludge as in the excess sludge. This highlights that although the amount of estrogens removed with sludge discharge is only a fraction of the amount entering a STP, adsorption to sludge within a plant contributes to a large extent to retaining estrogens inside the STP, so that the retention is long enough for biological degradation.

An exception is the Shiga activated sludge treatment system, treating nightsoil/septic tank sludge in Japan, where 99% of the estrogens are removed form the liquid phase during treatment, and 30% of the incoming estrogens are discharged with excess sludge (Takigami et al. 2000).

6.9. *Parameters influencing removal*

It is difficult to actually compare the different parameters using the available data set of full scale plants as treatment conditions at studied STPs are often not fully described. Also different sampling strategies and method of analysis have been used resulting in different conclusions. Also, batch experiments can not directly be translated to full scale plants as they are often spiked with a high concentration, which may be select for an adapted microbial population that would not develop under normal conditions and makes interpretations to full scale STP difficult (Snyder et al. 2001). Also batch tests are likely to overestimate true biodegradation rates, as they are carried out under ideal circumstances. Alternatively, the high estrogen concentrations in batch experiments might have a toxic effect on the sludge, especially as nitrifying bacteria are very sensitive, and therefore in some of the experiments inhibition might have occurred, resulting in a lower degradation value as would be found under full scale conditions, where the estrogen concentration is in the ng/l range. This inhibition is supported by the results of different researchers who always found a higher *k*-value in case of lower estrogen concentration, however nobody has concluded direct inhibition. Most of the experiments were carried out at 20 °C, whereas field conditions will be more frequently in the 10–15 °C range (Johnson & Sumpter 2001). Also in batch experiments, there is no oxygen depletion, while in activated sludge systems, anaerobic areas can appear.

Parameters influencing the degradation can be divided into parameters connected to climate, like rainfall and temperature and parameters connected to the design of a STP, like the HRT, SRT and the type of system, including the different treatment steps applied. As the latter has been discussed in detail in the previous chapter, the remaining parameters will be discussed below.

6.9.1. *Parameters related to climate*

The influence of rainfall is shown by Shore et al. (1993), where the concentrations of natural estrogens in the effluent were 7.5-fold higher in a drought year compared with a wet year, and removal percentages were ranging from 20 to 64% in the dry year and 88% in the wet year. This was also observed between samples collected in August, when the amount of rain has been substantial, compared to samples collected in May and April (Kirk et al. 2002). Apparently the higher influent concentration has a larger influence on the removal than the increased HRT, which can be expected as less wastewater enters the STP.

During winter, higher effluent concentrations for both natural and synthetic estrogens have been observed (Tabak et al. 1981; Desbrow et al. 1998; Belfroid et al. 1999a). This can be due to a temperature effect, since biomass is less active at lower temperatures. In case slow growing specified bacteria are playing a role in removing estrogens, especially for EE2, the reduced removal in winter can also be related to the reduced amount of these type of micro-organisms due to washout in winter-periods. The influence of temperature on the degradation of estrogens was also demonstrated in an activated sludge treatment plant for municipal sewage in both Germany and

Brazil (Ternes 1998) and six activated treatment plants near Rome in Italy (Baronti et al. 2000). The average air temperature was 20 °C for Brazil and −2 °C for the German plant, the temperature of the Italian plants is not known, but presumably in between the Brazilian and German temperatures, concluding from the periods, which are October to March for Italy, excluding January and November for Germany. The samples from Brazil and Germany are flow proportional composite samples, the values from Italy are calculated with grab samples, but obtained as the average of five sample times at six different plants (30 points in total). The removal percentages were in order of increasing temperature, so first German, and then Italy followed by Brazil, 14, 59 and 83% for E1, 64, 87 and 99.9% for E2 and −50, 80 and 78% for EE2. Only the hydraulic retention time (HRT) for the plants in Italy are known, which are 12–14 h, it can only be assumed that the HRT of the other plants are in the same order of magnitude. No information is available on sludge retention time (SRT), which also may have a significance impact on the removal efficiencies. For the plant in Germany, values for the effluent of the primary clarifier were given including the fraction sorbed on suspended solids, and the concentrations for all three estrogens were higher than in the total concentration in the influent. This illustrates insufficient deconjugation in the sewer, which also might be temperature related. If values are solely calculated for the biological activated sludge step, removals are 51, 76, −33% for E1, E2 en EE2, respectively. With the available data so far no correlation between the temperature and the E2 removal in full-scale treatment plants has been found (Johnson et al. 2000).

6.9.2. *Parameters related to design*

Longer hydraulic retention times give higher removal efficiencies of E1, E2 and EE2 as illustrated by STPs in the UK, at which removal is significantly better at an HRT of around 13 h compared to 2–5 h (Kirk et al. 2002). This is confirmed by Svenson et al. (2003), reporting removal below the detection limit for the Kävlinge plant with an HRT of 20 h and the Ekebyverket plant including a wetland with an HRT of 7 days (see Table 7). Approximately 99% removal was achieved in the Vimmerby plant with an HRT of 12 h, which was longer than the 2–8 h applied in most other plants in this research, only removing about 58–94%. Cargouët et al. (2004) found better removal for E1 (58%) and E2 (60%) in the plants Evry and Valenton with an HRT of 10–14 h compared to a plant in Achères with an HRT of 2–3 h in which a removal of 44% for E1 and 49% of E2 was established. In the plant containing three biolfilters including nitrification and denitrification in Colombes with an HRT of 2.5–4 h, 55% of E1 and 43% of E2 were removed. In all the four plants removal for EE2 was approximately 40%.

The influence of increased SRT is illustrated by a STP in Wiesbaden 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 (Ternes et al. 1999a), while at the increased SRT a reduction of around 90% is established in the full scale plant, which can indicate the growth of micro-organisms capable of degrading EE2 (Andersen et al. 2003). So below a certain SRT, degradation of EE2 will not occur.

Joss et al. (2004) stated the hypothesis that sludge loading is a key parameter influencing the removal of estrogens, as they found a lower degradation in the first compartments of monitored reactors. This means that micro-organisms would prefer to degrade other organic compounds above estrogens. However, no clear correlation can be found within one STP with different organic loadings in relation to the removal of estrogens. Johnson et al. (2000) tried to find a correlation between the flow per head and the E2 removal. Using the data from Svenson et al. (2003) to find a correlation between the percentage 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.

7. Fate in advanced/tertiary treatment

7.1. *Coagulation and activated carbon*

As has been observed at full-scale treatment plants, coagulation did not have any enhancing

effect on the removal of estrogens, which is also tested in batch-tests by the addition of ferric chloride (5–30 mg/l) to a 15 ng/l E1 solution at different pH values (5–9) leading to no removal of E1 (Ong et al. 2001). Also adsorption by iron phosphate precipitates would be unlikely to sorb large quantities of steroid estrogens (Johnson et al. 2000).

Powdered Activated Carbon (PAC) has an adsorption capacity between 2–62 ng/mg for E1 applied at concentrations of 3.6–65 ng E1/l (Ong et al. 2001). The adsorption of E1 is linear in a buffer solution, whereas using surface water and STP effluent it is not due to a preloading with other organics adsorbing to PAC as well. In a buffer solution with a concentration of 100 ng E1/l, a concentration of 5 mg/l PAC was removing more than 80% of the E1, and at 20 mg/l more than 95%, whereas for surface water containing E1 100 ng/l 80% removal was only achieved at a PAC concentration of 50 mg/l and for STP effluent it was not achieved at this concentration. As a post-treatment system emphasising on the removal of estrogens, the use of PAC may not be suitable, as a lot of PAC will be needed to achieve a sufficient removal. Also Fuerhacker et al. (2001) concluded that the adsorption of E2 to Granular Activated Carbon (GAC) is insufficient as at equilibrium, only 49–81% of the E2 in the 1–100 ng/l range is adsorbed in deionised water.

7.2. *Membrane filtration*

Membrane filtration processes include microfiltration (MF, macropores > 50 nm), ultrafiltration (UF, mesopores 2–50 nm), nanofiltration (NF, micropores < 2 nm), reverse osmosis (RO, dense < 2 nm), dialysis, and electrodialysis (ED) (Metcalf & Eddy 2003). NF distinguishes itself from RO as it only retains multivalent ions, so it has an economic advantage when the retention of monovalent ions is not required (Schäfer et al. 2003). The most important way to remove estrogens with membrane filtration is by retention on the membrane or by adsorption to organic particulates, since membrane pores are still larger than the radium of for example E1, which is 0.84 nm, while the average pore radium for a 1000 Da membrane is 0.94 nm (Schäfer et al. 2002b). The adsorption capacity of the membranes for hormones could be affected by membrane types, pH, affinity of hormones to water, as well as the presence of other organics (Chang et al. 2002b). Estrone retention is higher in presence of organics (Schäfer et al. 2002b;Schäfer & Waite 2002), since the compound is attached to the organic, which is retained by the membrane. Therefore, adsorbents used during wastewater treatment as powdered activated carbon, ferric chloride coagulant and Magnetic Ion Exchange Resins (MIEX®) have been investigated (Schäfer & Waite 2002). This research confirmed again that the addition of ferric chloride does not change the E1 concentration. With a relatively low dose of activated carbon (5–10 mg/l) over 80% of removal of E1 is achieved. MIEX® can bind small organic pollutants and polar compounds. Adding MIEX® the removal of E1 is up to 45% and increases with pH. When the molecules are dissociated at a pH above 10.4, the removal is up to 70% (Schäfer et al. 2002a; Schäfer & Waite 2002).

A number of commercially available NF and RO membranes have been investigated for the retention of E1 dissolved in carbonate buffer (Schäfer et al. 2003). In general the retention at an initial concentration of 100 ng/l was very good, 95–99% with the exception of one, which was 80%. For used membrane types, both size exclusion and adsorptive effects are responsible for maintaining high retention of E1 in this type of membrane. Adsorptive effects appear to be particularly important for retention by NF membranes exhibiting relatively low ion retentions. These adsorptive effects may be driven by hydrogen bonding between E1 and the membranes. Deprotonation leads to a significant decrease in retention, possibly as a result of a critical role of the hydroxyl-group or as a result of strong electrostatic repulsive forces (Schäfer et al. 2003).

Even with microfiltration or ultrafiltration, pore sizes are too big and the main removal mechanism will be adsorption to the membrane, which is low at neutral pH and decreased at pH higher than 10.5 (Schäfer & Waite 2002). The adsorption on hydrophobic membranes is higher then on hydrophilic material. To determine whether, in case of NF membranes, E1 is removed by size exclusion or by adsorption, the pH can be increased above 10.5. Since E1 is dissociated and charged it won't adsorb to a negative charged membrane and if the removed amount stays

the same, size exclusion is the main removal mechanism.

In an attempt to use antigens in a porous hollow fibre membrane for the removal of E2, breakthrough was depending on the ligand density in the membrane, which was at most 5.8 mg/mg glucidyl methacrylate fibre (Nishiyama et al. 2002). The molar binding ratio was very low (0.043) and the amount of E2 bound to the fibre decreased with an increasing number of cycles, which might indicate that the binding capacity is decreasing, possibly by the use of the eluent fluid (10% methanol/water). It is clear that insufficient data are available for any application in the current state.

Microfiltration had been compared to a microfiltration PAC hybrid system for the removal of E1 from a buffer solution (Ong et al. 2001).Without the addition of PAC, the membrane has been saturated after approximately one hour and shows a decrease in E1 retention, while with the addition of PAC the removal is a function of PAC concentration, higher concentration leads to a faster equilibrium.

The amount of sorption of E1 was researched for different types of membranes, different pH values, ionic strength and competition by other organics (Chang et al. 2002b). It was concluded that E1 has a higher affinity for hydrophobic membranes. There was not much difference between an ionic strength of 0.02 and 0.2 M and the pH only has an influence above pH 11, as the molecules become charged, lowering the affinity for the membrane, since they are both negatively charged. There was competition with other organic materials, since E1 removal in a buffer solution showed higher removal compared to E1 removal in surface water and secondary effluent, although the removal was not influenced dramatically. The retention on the membrane decreases with the increase in the surface concentration and a breakthrough will occur when the surface concentration reaches the equilibrium value for the corresponding feed concentration (Chang et al. 2002a, b).

When using a membrane in combination with an activated sludge system, the so-called membrane bioreactor, enhancing effects on the removal of estrogens could be expected because of an increased biomass concentration and longer SRT (Wintgens & Melin 2001). As far as we are aware there are no data available for this particular process regarding the removal of E1, E2 and EE2.

7.3. *Ozonation and advanced oxidation processes*

Ozonation and especially Advanced Oxidation Processes (AOP) are used to convert complex organic materials in wastewater so they lose their toxicity (Metcalf & Eddy 2003). AOP uses hydroxyl free radicals ($HO^{\cdot}$) as nonselective oxidant. And they are formed by using ozone and UV, or ozone in combination with hydrogen peroxide or hydrogen peroxide in combination with UV. In a few cases AOP is tested for the conversion of estrogens in final effluents as described below.

Even after tertiary treatment of municipal wastewater effluent, including sand filtration, ozone and UV, E2 and E1 are still detected in the effluent in a concentration of 0.8 and 1.3 ng/l, respectively, while EE2 stays below the detection limit of 5 ng/l in both influent and effluent (Chapman 2003). So separate ozonation might not be as effective as AOP. There are no further specifications given about the treatment steps itself, so it is difficult to draw a conclusion from this research. Applying 10–15 ng/l ozone with a contact time of 18 min, it is able to remove E1 in a concentration of 0.015 μg/l to bellow the detection limit from STP effluent from an activated sludge plant treating municipal wastewater in Germany (Ternes et al. 2003).

E2 is highly reactive towards ozone because of the two reactive hydroxyl groups (Kosaka et al. 2000). During a treatment with O_3/H_2O_2, ozone is more selective than $HO^{\cdot}$, and since E2 is a highly reactive target, it will be removed quite easily even in the presence of radical scavenging compounds such as humic acid (Kosaka et al. 2000). Also EE2 can be attacked by ozone and the halflife time was 5 μs under typical ozonation conditions (Gunten et al. 2003; Huber et al. 2003).

In a study comparing sand filtration, ozone/hydrogen peroxide (AOP), micro filtration and reverse osmosis for the removal of estrogenity from municipal wastewater, only AOP and reverse osmosis were able to remove total estrogenic activity for over 97% while in the other options, the removal was insufficient (Shishida et al. 2000).

8. Conclusions

From the three studied compounds, E1, E2 and EE2, the mineralisation rate of EE2 was considerably lower compared to E1 and E2, while sorption of EE2 to sludge is higher compared to E1 and E2. Mineralisation rates are higher in a temperature range from 20 to 25 °C compared to 5–10 °C. Sludge with a higher SRT shows a faster degradation of E1, E2 and EE2. The phenomenon of increased degradation rates at lower initial concentration should be looked at in more detail in order to investigate a suitable explanation.

The adsorption constants for different types of sludge would be useful to determine for the aid of mass balances for full scale plants. The constants can be determined by measuring the estrogens adsorbed on sludge and dissolved in the water phase separately. To exclude biodegradation and photolysis it is recommended to perform the test with inactivated sludge in the dark. Inactivation should not lead to a change of the sludge structure, because it can have influence on the binding places.

Although the risk for bioaccumulation of natural estrogens from domestic wastewater in the environment is expected to be small, the synthetic hormone EE2 however, expected risks for accumulation can be significantly higher, due to their slow degradation. In order to prevent estrogenic effects on fish, especially in cases where there is little effluent dilution, the improvement of STPs regarding the removal of estrogens will be the only long-term solution. Another issue that deserves some thinking over is how to deal with the sludge. During anaerobic sludge treatment little or none of the estrogens is removed. In case sludge is used on (farm)land, estrogens might run-off with the rainwater and end up in streams.

Although activated sludge is able to biodegrade estrone, 17β-estradiol and 17α-ethynylestradiol, they are still present in final effluents in the ng/l range. In order to obtain more fundamental knowledge on removal processes, more attention should be paid to parameters that can influence the conversion rate of estrogens, like the HRT, SRT and loading rate. This knowledge can contribute to an optimisation of existing treatment plants, rather then addition of extra tertiary and costly treatment steps. Another important research area is the development, standardization and validation of determination methods for estrogens in the wastewater and sludge matrix. As there is no standardised method in order to measure E1, E2 and EE2 in the wastewater and sludge, it would be advantageous to introduce such a method to ease the comparability of different researches. Also there seems to be a lack of research for ''real'' wastewater, especially in the cases of tertiary treatment.

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