Estrogenic compounds, an emerging aquatic pollutant: an overview of their measurement and treatment methods

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Abstract

Estrogens are hormonal secretions of humans and animals. Their natural degradation cycle is disrupted when the populations of both humans and livestock increases at a tremendous rate, increasing the amount of estrogen released into water bodies. Pollution of estrogenic compounds has caught the attention of researchers as the slight increase of estrogens in the water bodies has significant impact on the aquatic system. In this review, we compare various estrogenic compound analysis methods, identify sources and levels of estrogenic hormonal secretion pollution in surface as well as sewage waste waters and discuss various treatment technologies used to remove estrogenic compounds. We found that the use of different analysis methods hinders an accurate comparison of data. A comprehensive review is conducted on diverse removal technologies, identifying their advantages and disadvantages, followed by recommendations on strategies to deal with estrogenic pollution in water bodies.

Introduction

Estrogen is categorized under steroid hormones. In mammalian system, steroid hormone is classified into six major groups, namely, glucocorticoids, mineralocorticoids, androgens, estrogens, vitamin D, and progestogins.[1] Steroid hormones work by binding to a receptor on a plasma membrane, such as the Lock-and-Key Model of hormone receptor, to further trigger other biochemical activities.[2]

The dominant natural hormones in females are estrone (E1), estradiol (E2), and estriol (E3).[3] The properties of these estrogenic compounds are shown in Table 1.[4-6] Estrogen is essential for pregnancy, sexual reproductive tissue maintenance, cell differentiation, and growth.

Synthetic estrogen derivatives in medicine are those of the C-17 group, such as EE2, MeEE2, and Quinestrol. Synthetic hormones are typically synthesized to be used as a birth control pill and hormone replacement therapy for breast cancer or prostate cancer treatment. All types of estrogen including natural or synthetic estrogen are moderately toxic, with 0.5 to 5 g kg^{-1} as the probable oral lethal dose for humans.[7]

Past studies have proven the presence of estrogenic compounds in surface water.[8-11] Estrogenic pollutions as such are usually due to animals and human excrements, indicating that the current available conventional treatment technologies are not effective enough to completely remove these estrogenic compounds. Thus, studies have been conducted to improve the current treatment system in removing estrogenic compounds. Methods developed ranged from conventional activated sludge system, to biological treatment using pure culture,[12] and chemical treatment.[13-15]

Although many studies have been performed on the effects of estrogenic compounds pollutions on the environment along with various methods of treatment of these compounds, reports that contain comprehensive review on the subject are almost non-existent. Hence, in this paper, critical comparisons are being made on various technologies that are employed to treat water containing estrogenic compounds as well as on different methodologies used to analyze the compounds. In addition, suggestions are being made on viable methodologies to treat water containing estrogenic compounds effectively.

Of all the reviewed treatment methods, only physical/chemical treatment with manganese oxide[16] and sorption with activated carbon[15] could achieve a removal of 100%. Highest possible removal is vital as there is no minimal safe limit that has been establish till date. However, such treatment does not usually resolve the estrogenic pollution from cradle to grave. These treatment techniques merely temporary remove these contaminants from one source to another. In contrast, biological treatment has the ability to transform the estrogenic compounds from molecular structure level and thus degrading it. However, the retention time required for biological treatment process usually range from 10 to 100 days. Thus, a combination technology of both to ensure the effective removal and solution to root may be employ.

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Effect of estrogenic compounds and sources in water bodies

The presence of estrogenic compounds in our aquatic systems has been proven by several studies.[3,9,17-19] The concentrations of estrogen released in the sewage system range between 10 to 100 ng L^{-1}, whereas the efficiency of their removal from sewage treatment only range from 50 to 95%, before being discharged into the river line.[12] Thus, several studies on the effects of estrogen to aquatic organisms have been conducted. Estrogens in the environment cause the adaptation of aquatic organisms to the exposure by modifying their characteristics, such as female gonadal phenotype, decrease in fertility, and fish feminization.[6,17,20-22]

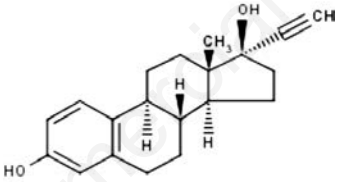
One of the most drastic examples of the effect of estrogen was reported in a study conducted over a three-year period on fathead minnow fish. The study started out with 7000 fish before the addition of EE2; the fish community was almost completely wiped out after 3 years of study. This phenomenon was due to kidney failure, tissue death in the testes, immature fish with little

or no sperm for male fish, and immature egg for female fish.[23] Compared with other EDCs found in wastewater, estrogens have three to sevenfold greater estrogenic potency. The general prediction of maximum concentration that causes no effect is 1 ng L^{-1} for E2 and 3 to 5 ng L^{-1} for E1.[24]

For humans, the increasing rate of breast cancer and certain anomalies in the reproductive system have been attributed to estrogenic exposure, even at small concentrations.[25,26] Sources of estrogenic compounds are from contraceptive pills used for birth control, hormone treatments, such as growth promoter; induced abortions; muscle building; estrous cycle of farm animals, and discharges of humans and animals that end up in sewage treatment plants.[4,12,17] Thus, wastewater treatment plants have become the cumulative center for estrogenic compounds which are subsequently released in water bodies after treatment.[11]

Discharges from farm animals, such as cow, sheep, swine, and goat, have steroid hormones with a concentration range of 14 to 533 ng g^{-1} dry waste, whereas a typical range of 44 ng g^{-1} was reported for E2.[4] Excretion of farm animals according to their groups is shown in Table 2.[27] The amount and proportion of estrogen excreted by each individual organism vary. Majority of estrogen discharged from cattle are in feces (58%), whereas that in swine and poultry, the discharge is mostly in urine with 96 and 69%, respectively.[19,28] Discharge of estrogenic compounds in all organisms also varies at different stages of their maturity, pregnancy, and lactation. The use of manure fertilizer can also contribute to estrogenic activity in surface water[20] because the half-life of estrogenic activity in manure fertilizer in soils takes up to 5 to 25 days, whereas sheep and cattle manure of different ages will take 7 to 2 years.

Water runoff and leaching also cause contamination of freshwater supply.[28] In the US, the overall hormone discharge has been estimated at more than 330 tons $year^{-1}$. According to Zhao and Zhang, only 0.003% of the total amount of estrogen excreted will eventually end up in rivers. Although animal wastes are often applied in agricultural plantations, the high manure to land ratio often results in their disposal because the waste produced are way above the needs of the plantations.[29] Meanwhile, the amount of estrogenic hormones excreted by each individual per day, with the pregnant woman producing the highest contribution of estrogen compound to wastewater. The males and menopausal females have the lowest excretion. On the average, 10.5 µg d^{-1} of E1, 6.6 µg d^{-1} of E2, 3.3 µg d^{-1} transformation of E1 to E2, and 1 µg d^{-1} of EE2 are excreted by humans per individual.[30] Values are reported in Table 3.

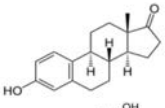
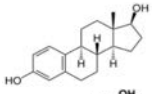
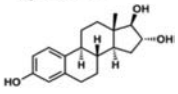
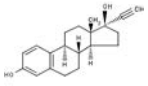
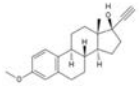


Methods to determine estrogenic concentration

Currently, there is no particular worldwide-accepted standard to determine the estrogenic compounds concentration in water bodies. Most analytical methods used in past studies include HPLC-based,[8,33] GC-based[29,34] and *vitro* bioassay. [25,35-37] HPLC-based and GC-based analytical techniques are used in combination with mass spectrometry. Most of these analyses involve a pre-analysis procedure of solid-phase extraction or liquid-liquid extraction methods. Water sample is extracted into a medium, and then eluted for analysis. According to the Environmental Protection Agency (EPA), GC-based is the standard procedure for hormone identification under the Clean Water Act (CWA) (U.S. Environmental Protection Agency 2007). This standard is however, not employed by most researchers because the use of high-resolution GC combined with high-resolution mass spectrometry (HRGC/HRMS), which are required in the standard, is not available in most research labs. Thus, HPLC and bioassay are the most frequently used methods. In addition to these conventional analysis methods, complementary methods such as liquid-chromatography, electrospray, and atmospheric pressure photoionization have been developed to analyze estrogenic compounds.[38] Table 4 shows the estrogenic pollution levels in various water bodies, along with the method used for analysis.

Various methods are used for the analy-

Table 1. Properties of estrogenic compounds.

Type of estrogens	Name	Structure	Molecular weight	Solubility (mg L^{-1} at 20°C)	Vapor pressure	*In vivo* vitellogenin response in trout, (mm Hg)
Natural	Estrone (E1) $C_{18}H_{22}O_2$		270.4	13	2.3×10^{-10}	0.5
	17β-Estradiol (E2) $C_{18}H_{24}O_2$		272.4	13	2.3×10^{-10}	1
	Estriol (E3) $C_{18}H_{24}O_3$		288.4	13	6.7 x 10-15	-
Synthetic	17α-Ethynylestradiol (EE2) $C_{20}H_{24}O_2$		296.4	4.8	4.5×10^{-11}	25
	Mestranol (MeEE2) $C_{21}H_{26}O_2$		310.4	0.3	7.5×10^{-10}	-

EEQ, estrogen equivalent concentration.

sis of estrogenic levels and for water sampling. Most researchers employ a method of acidifying the sample before transporting it for analysis.[6,11,17,18,35,39,50] Some transport the water sample in its original form without any preservation procedure,[46,48,51] whereas others preserve the samples in formalin[19] or formaldehyde[8] to prevent bacterial degradation of natural steroids.[32] However, estrogenic compounds have weak acidity; thus, samples need to be acidified for better retention during the solid-phase extraction processes.[8] The method used by EPA requires the immediate adjustment of the pH of the samples to pH 2 with concentrated sulfuric acid after sampling.[52]

Levels of estrogenic compounds in water bodies

Data of estrogenic pollution were obtained from river water, sewage treatment plants, and wastewater treatment plants. Samples from the reservoir of water catchment areas, which are at their purest state, are yet to be studied. The presence of estrogenic compounds was confirmed and their levels were determined in several countries, as shown in Table 4. The overall levels were surprisingly high. France had a significantly high value of 78.8 ng L^{-1} for E1, 23.7 ng L^{-1} for E2, and 313 ng L^{-1} for E3.[41] In Malaysia, 6.5 ng L^{-1} for E1, 2.3 ng L^{-1} for E2, and 8.6 ng L^{-1} EE2 were detected in Sabah surface water,[17] whereas those in Kuala Selangor, Selat Kelang, and Sungai Buluh are 2.6, 63.0, and 61.7 ng L^{-1}.[10] These values were high enough to cause modification in our aquatic ecosystems, particularly gender characteristics.

In 2002, Ying and Kookana reported that E1, E2, and EE2 in the European continent were less than 5 ng L^{-1}.[4] This report differs with most studies conducted in 2010, which recorded values as high as 313 ng L^{-1} for E2.[41] Most of the data were recorded in Italy.[4,47] This indirectly showed an increasing trend of estrogenic contaminant in surface water over the years.

From the reported data, the recorded values for E1 and E3 were higher than those of E2[4,11,41] High levels of E1 and E3 were found in the water because they were the major metabolites of E2 and EE2, which will degrade slowly to E3.[8] In addition, the higher E1 values compared with E2 were due to the oxidation of E2 to E1.[32,48] The transformation process of E2 to E1 usually has a half-life of 0.2 to 9 d when incubated at 20°C.[53] E1 and E2 takes up to 98% of the total estrogenic activity in wastewater effluent.[24]

The development of dairy industries in New Zealand caused pollution in nearly all of their catchment areas.[19] The detected level of 17α-E2 in New Zealand was higher compared with other types of estrogen, with a value of 730 ng L^{-1}. This result was ascribed to 17α-E2, which was the dominant form excreted only by cattle; other livestock or humans do not excrete estrogenic component in this form.[19,35] However, 17α-E2 is safer compared with 17β-E2 because it is a less feminizing isomer and its potential toxicity is lower.[54]

The values obtained in Queensland, Australia showed an increasing trend of estrogenic levels in their wastewater treatment plant. E1 detected in Ipswich City was 9.12 ng L^{-1} in 2004. However, the value detected was 29.12 ng L^{-1} in 2005. The same trends were recorded for E2 and EE2 at 1.37 and 0.14 ng L^{-1}, respectively, in 2004, and 5.69 ng L^{-1} and 1.14 ng L^{-1}, respectively, in 2005. The treatment plants in Ipswich City, Logan City, and Brisbane City recorded a reasonably similar value of estrogen discharge at their effluent because all primary treatments were based on biological treatments such as activated sludge and biological nutrient removal.[6]

Concentrations of estrogenic compounds in river water vary based on distance travelled and its dilution effect, together with degradation rate. According to a report by Pawlowski (2004), the effluent point of EE2 at a sewage treatment plant marked a reading of 34.10±7.18 ng L^{-1}. However, the reading at its downstream dropped to 19.42±2.80 ng L^{-1}. The same goes for River Neckar where the effluent from the sewage treatment plant was 65.96±10.40 ng L^{-1} and the value at its downstream was 11.81±0.70 ng L^{-1}.[36]

Most of the results obtained were based on one-off studies, where the samples were collected at a particular duration of time for analysis, without taking into account seasonal changes. Only a few studies identified the fluctuation of data due to seasonal changes.[43,53] Decaying rate of estrogen varied with distance and season; however, no solid judgment was made because the velocity and transit time of water were not measured.[43] Fluctuation of data should take into account the metabolic rate of bacteria that increases and decreases throughout the season changes.[55]

Beck and Radke (2006) showed that aeration systems in wastewater treatment plants also cause the dispersal of estrogenic compounds to the atmosphere. The values obtained from aerated aerosol were 174±215 pg m^{-3} for E2 and 159±75 pg m^{-3} for E1.[45] In addition, a study from Poland detected 2.1 and 0.5 ng L^{-1} of E2 and EE2, respectively, in drinking water.[15]

As mentioned earlier, the use of different methods of analysis could result in inac-

Table 2. Excretions of estrogenic hormones by each farm animals (d^{-1}).

Group	Total estrogen (μg d^{-1})
Cattle	
Calves	45
Cycling cows	299
Bulls	540
Swine	
Cycling sows	120
Boars	2300
Sheep	
Cycling ewes	23
Rams	25
Chickens	
Female broilers	0.93
Male broilers	0.19
Laying hens	19.45
Cocks	3.29

Table 3. Excretions of estrogenic hormones by each person (d^{-1}).

Group	E1 (μg)	βE2 (μg)	E3 (μg)	References
Male	3.9	1.6	1.5	(31)
Women	20	5	64	(24)
Menstruating females	8	3.5	4.8	(31)
Menopausal female	4	2.3	1	(31)
Pre-menopausal women	2.66	1.09	5.68	(8, 32)
Pregnant women	600	259	6000	(31)

E1, estrone; E2, estradiol; E3, estriol.

Table 4. Levels of estrone, estradiol, estriol, and ethynylestradiol in different water bodies according to continent.

Continent	Country	Sample type	E1 (ng L^{-1})	E2 (ng L^{-1})	E3 (ng L^{-1})	EE2 (ng L^{-1})	Analysis method	References
Asia	Taiwan	River water	22.4-66.2	1.40-33.9	12.4- 73.6	7.53-27.4	LC-MS/MS-negative electrospray ionization	(8)
	Taiwan	WWTP effluent	10.2-48.6	4.5-44.5	ND-39.1	2.25-37.9	LC-MS/MS-negative electrospray ionization	(8)
	Taiwan	Hospital effluent	415	230	-	432	SPE/HPLC-MS/MS-positive electrospray ionization	(9)
	Taiwan	Pharmaceutical Production Facilities effluent	115	112	-		SPE/HPLC-MS/MS-positive electrospray ionization	(9)
	Japan	Sewage treatment work	0.39-10.49	1.35-9.05	-	0.59-6.56	SPE/ HPLC-MS – negative electrospray ionization	(39)
	Malaysia (Kuala Selangor)	Urban and recreation areas	2.4	0.2	-	-	SPE/assay	(10)
	Malaysia (Kapar)	Adjecent to coal-fired power plant	16.1	5.9	-	-	SPE/assay	(10)
	Malaysia (Sg. Buluh)	Fishing village	58	3.7	-	-	SPE/assay	(10)
	Malaysia (Selat Kelang)	Urban	57.3	5.8	-	-	SPE/assay	(10)
	Malaysia (Sg. Sepang Kecil)	Agricultural and fishing	10.5	4	-	-	SPE/assay	(10)
	Malaysia (Sg. Sepang Besar)	Agricultural and fishing	3.9	2	-	-	SPE/assay	(10)
	Malaysia (Kuala Lukut)	Agricultural	2.8	2	-	-	SPE/assay	(10)
	Malaysia (Kuala Linggi)	Agricultural and aquacultural	6.9	2.1	-	-	SPE/assay	(10)
	Republic of Korea	Influent municipal WWTPs	29	17	379	-	SPE/LC-MS/MS-negative electrospray ionization	(11)
	Republic of Korea	Effluent municipal WWTPs	19	-	206	-	SPE/LC-MS/MS-negative electrospray ionization	(11)
	Republic of Korea	Influent livestock WWTPs	3650	237	656	-	SPE/LC-MS/MS-negative e electrospray ionization	(11)
	Republic of Korea	Effluent livestock WWTPs	164	-	200	-	SPE/LC-MS/MS-negative electrospray ionization	(11)
	China	Influent WWTPs	8.7±7.5	1.5±1.5	-	-	SPE/LC-ESI-MS/MS	(33)
	China (Beitang River	River water	23.4	8.69	10.3	10.0	SPE/GC-MS	(32)
	China (Dagu River)	River water	19.7	10.3	12.4	9.45	SPE/GC-MS	(32)
	China (Yongding New River)	River water	10.5	7.26	5.76	3.54	SPE/GC-MS	(32)
	Japan (Manko Tidal Flat)	Wetlands	9.2	<1	-	-	SPE/LC-MS/MS	(40)
Europe	France	WWTP influent	78.8	23.7	313	-	SPE/LC-MS/MS	(41)
	France	WWTP effluent	8.2	4.2	33.5	-	SPE/LC-MS/MS	(41)
	France (Rhône-Alpes)	Surface water	0.3	-	-	-	SPE/LC-MS/MS-electrospray ionization	(42)
	France (Rhône-Alpes)	Ground water	3.5	1.3	-	3	SPE/LC-MS/MS-electrospray ionization	(42)
	France (Eysines)	STP	57.8±2.8	4.4±0.8	2.9±0.1	<2.0	SPE/GC-MS	(43)
	France (Upstream Acheres)	Surface water	1.1±0.3	1.4±0.6	1.5±0.5	1.5±0.5	SPE/GC-MS	(44)
	France (Downstream Acheres)	Surface water	3.0±0.9	3.0±0.6	2.5±0.6	2.9±0.6	SPE/GC-MS	(44)
	Germany (Bayreuth)	WWTP	2100±1000	2100±900	-	-	SPE/GC-MS	(45)
	Spain (Llobregat River)	River water	22	-	-	-	SPE/LC-MS —negative electrospray ionization	(46)
	Germany (River Neckar)	Effluent of STP	19	5.6	-	1.5	SPE/YES assay	(36)
	Germany (River Rhine)	Effluent of STP	1.2	1	-	<1	YES assay	(36)
	Italy	Condominium collecting tank	58	9	62	-	SPE/LC-MS	(47)
	Italy	Influent STP	44	11	72	-	SPE/LC-MS	(47)
	Italy	Effluent STP	17	1.6	2.3	-	SPE/LC-MS	(47)

Continued on next page.

curacy of reporting and comparing the levels of estrogenic compounds. Overall, the levels of estrogenic compound in the water bodies are still considered at a very high level. These levels have surpassed the general estimate of maximum amount that causes no effect that is 1 ng/L for E2 and 3 ng/L to 5 ng/L for E1.[24] Furthermore, examining the data reviewed over the years, the increasing trend of estrogenic pollution in water bodies should not be taken lightly. A minimal concentration of 1 ng/L of E2 and less than 0.1 ng/L of EE2 has been proven to interrupt the survival of aquatic organism.[24]

Treatment methodologies for the removal of estrogenic compounds

There are more than a handful of treatment methodologies that has been explored by the researchers over the years. They can basically be classified into two groups, that is the biological treatment and chemical/physical treatment. The review below will be engaged in two different directions, which is the treatment methods in actual and pilot plant and treatment methods in laboratory scale. Summary flow of both directions that will be discussed in Figure 1.

Conventional treatment methodologies

The most common treatment method for the removal of estrogenic compounds is the biological treatment system. The common practice systems discussed in Table 5 are based on biological systems such as activated sludge. In Gatton Shire, Australia, which is located at a rural area where high input of animal waste is possible, trickling filters with chlorine are used. Meanwhile, in Beaudesert Shire, Australia, which has the lowest value of estrogenic activity, biological filters with anaerobic solid digestion and chlorine disinfection are applied in wastewater treatment. However, biological filter technology is less effective compared with activated sludge due to its shorter hydraulic retention time.[6]

In a US livestock farm, removal of estrogenic compound in manure was obtained by storing and retaining manure in lagoons for 8 months to degrade 99.8% of estrogen. Laboratory scale tests proved that a near 100% degradation of estrogen took place after a mere four week.[61] In addition, the rapid degradation of EE2 in particular took place in aerobic conditions and at a slower rate for natural estrogen.[29,60] Several researchers have also proven that there is no degradation of EE2 during anaerobic or anoxic condition.[60,62,63] Variation in degradation rate of estrogenic compounds is due to the different conditions at each reported location and surrounding. Different temperatures, flow rates, dilution factors, water mixture compositions, and the types and amount of microorganisms affect degradation rate. Even after primary treatment on estrogenic compounds, there may still be a possible increase in the concentration detected because the existing conjugated estrogens are cleaved to free estrogens.[57]

In typical wastewater treatment plants, biological treatment systems such as sorption and biodegradation in activated sludge systems are usually used in removing estrogens. The sequence of easy sorption usually follows E3> EE2> E1> E2. Although sorption of estrogen takes place at a fast pace, the main step in estrogen removal is biodegradation. The mechanism of biodegradation includes deconjugation, degradation by heterotrophic bacteria, co-metabolism with nitrifying biomass, and other co-metabolisms. Activated sludge can remove 44 to 99.9% of E2, 18 to 100% of E3, up to 98% of E1, and 34 to 100% of EE2. However, favorable results can only be achieved in plants with SRT of more than 10 d, and not in highly loaded plants.[24] This result was supported by Oishi and Moriuchi, who reported that E2 and E1 in stream water have half-lives of 0.1 and 11 d, respectively.[14,64] Similar to another study, 17β-E2 was reported to have a biodegradation time in the water matrix of 3 d to 27 d; 17α-EE2 has 46 d.[15]

Adsorption of E1, E2, E3, and EE2 into the activated sludge system is an exothermic process, and its adsorption capacity decreases with the increase of temperature.[64] Adsorption capacity of activated sludge processes is dependent on temperature, sludge age, hydraulic retention time,

Table 4. Continued from previous page.

Continent	Country	Sample type	E1 (ng L^{-1})	E2 (ng L^{-1})	E3 (ng L^{-1})	EE2 (ng L^{-1})	Analysis method	References
Oceania	New Zealand	Farm dairy shed effluent	100 (10-580)	24 (3-310)	-	85	SPE/GC-MS	(35)
	Australia (South East Queensland)	WWTP effluent in ipswich city	29.12±0.54	5.69±0.51	-	1.14±0.32	SPE/assay	(6)
	Australia (South East Queensland)	WWTP effluent in logan city	21.33±2.06	3.73±0.11	-	0.57±0.02	SPE/assay	(6)
	Australia (South East Queensland)	WWTP effluent in brisbane city	25.77±0.41	6.35±0.14	-	1.20±0.04	SPE/assay	(6)
	Australia (South East Queensland)	WWTP effluent in beaudesert shire	17.64±0.58	3.60±0.35	-	0.75±0.03	SPE/assay	(6)
	Australia (South East Queensland)	WWTP effluent in Gatton shire	32.17±3.89	4.71±0.09	-	0.71±0.01	SPE/assay	(6)
	Australia (Malabar, Sydney)	STP	54	14	-	<5	SPE/GC-MS	(30)
North America	United State (Oklahomam)	Swine lagoon	9940	194	6290	-	SPE/GC-MS/MS	(48)
	Canada (Thames River)	WWTP	29.5	8.3	-	-	SPE/GC-MS	(49)
South America	Brazil (Rio de Janeiro)	River water	-	-	3.68	-	SPE/LC-MS/MS	(18)

E1, estrone; E2, estradiol; E3, estriol; EE2, ethynylestradiol; SPE, solid phase extraction; LC-MS, liquid chromatography mass spectrophotometry; GC-MS, gas chromatography mass spectrometry; YES, yeast estrogen screen.

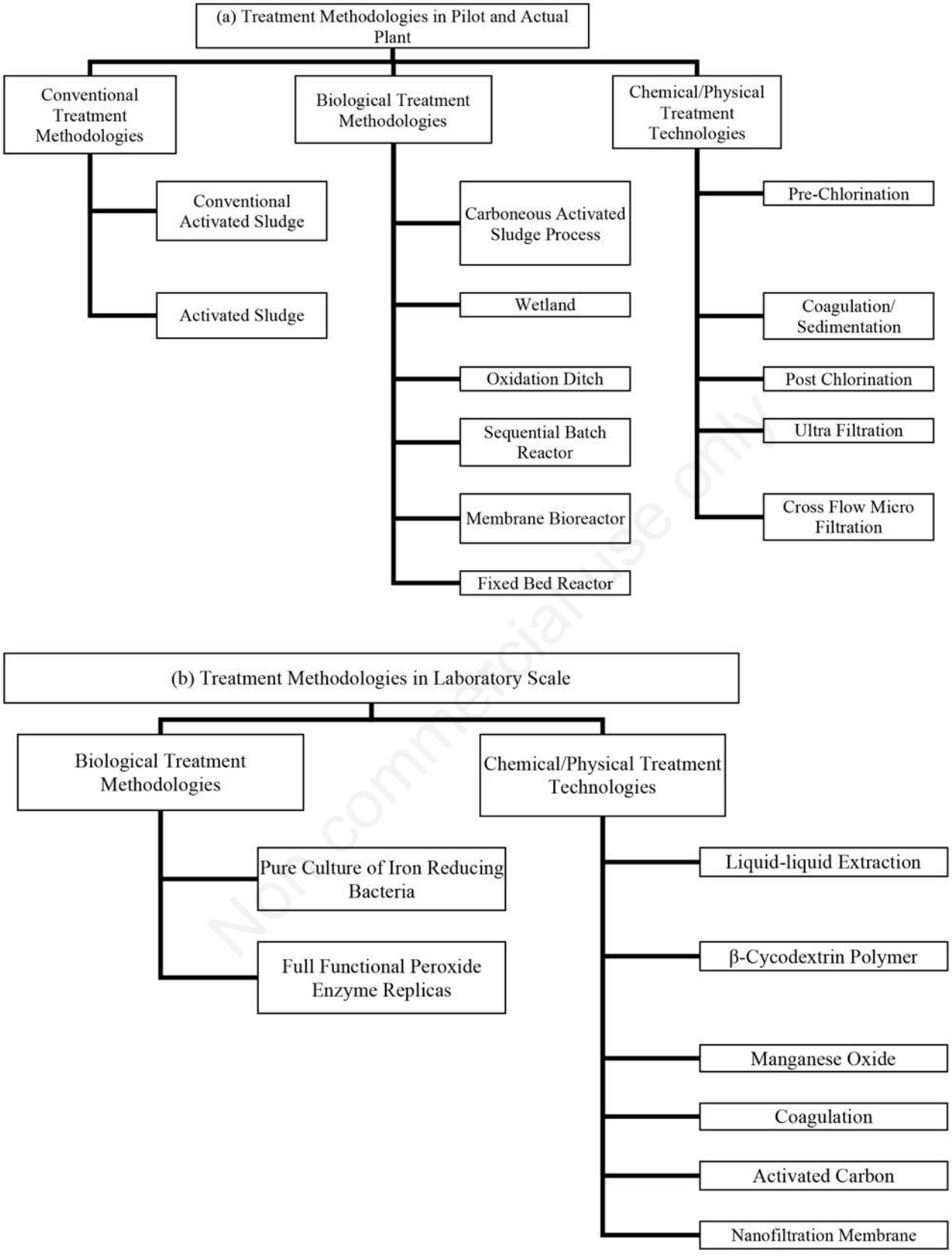


Figure 1. Treatment methodologies in (a) actual and pilot plant and (b) laboratory scale.

sewage composition,[65] and dilution of wastewater.[31] However, findings from Anderson and Hansen (2005) showed that excess sludge used in activated sludge system is not significant because only 2% of the total estrogen component adsorbed in the sludge will be removed as an addition to the 50% to 75% adsorption into the exact amount of sludge used.[66] This result indicates that removal and degradation of estrogenic compounds in an activated sludge system are mainly ascribed to the bacteria degradation activity in the liquid phase. Removal of estrogen naturally occurs by conjugate transformation in the treatment process.[11]

Although several studies have been conducted on conventional wastewater treatment plants where activated sludge systems partially remove and degrade estrogenic compounds, their sorption constant, mechanism, and interaction pattern were not thoroughly discussed.

Other treatment methodologies

The conventional method of wastewater treatment can remove only 80±19% of βE2, 67±51% of αE2, and 76±46% for E1 compared with 100% removal for androgens and progestogens. The lower removal rates are due to the presence of benzene rings in estrogens, which are harder to degrade.[33] The inefficiency of wastewater treatment plants in removing steroids was recorded in the 1960s.[50] Table 5 shows various treatment methodologies employed by pilot scales or actual plants in the removal of estrogens, along with their performance in terms of percentage removal. Table 6 reports some laboratory scale studies along with their performance.

Tables 5 and 6 show that the removal of estrogenic compounds in biological treatment exhibits a trend of better removal in E2 and E3 compared with E1. Regardless of the retention time, a removal of 80% and above is possible for the biological treatment. However, laboratory-scale study proved that other methodologies significantly and effectively removed all estrogenic compounds.

The most effective treatment reported thus far is treatment with activated carbon and manganese oxide,[8] as shown in Table 6. Removal of estrogenic compounds by manganese oxide is effective and fast. The removal rate can achieve 60% in the first 20 min and a near complete removal within

Table 5. Percentages removal of estrogenic compounds by different treatment methodologies in pilot and actual plant.

Method		E1%	E2%	E3%	EE2%	Additional notes	References
Conventional biological treatment	CAS treatment in Kanto region, Japan	-21-68	83-98	99.5	-	-	(56)
	CAS treatment at Altenrhein	49±15	88±9	-	71±9	-	(57)
	CAS treatment at Kloten	96±1	>97	-	94±2	-	(57)
	Activated sludge sewage treatment plants in Italy	61	87	95	85	-	(58)
Biological treatment	Carboneous activated sludge process	73±29	56±17	-	-	EE2 partition coefficient was found lower at log Kp 0.5	(59)
	Wetland attached to WWTP	67.8±28.0	84.0±15.4	-	75.3±17.6	Low wetland depth improves the estrogen removal	(39)
	OD in WWTP	83.4	94.3	98.9	-	SRT of about 100 d	(51)
	Sequential batch reactor	85	96	-	-	Mass balance shows 25% of total mass load of E1 and E2 accumulates in mixed liqour suspended solids	(30)
	Sequential batch reactor from city of Puyallup, Washinton WWTP	-	-	-	*72-93	Seeded with 10 days SRT Activated sludge	(60)
	Sequential batch reactor from Durham Oregon WWTP	-	-	-	*>90	Seeded with 11 days SRT mixed liquor	(60)
	Sequential batch reactor from King Country, Renton WWTP	-	-	-	*76-99	Seeded with 13 days SRT mixed liquor and duration of aerobic SRT treatment for removal was 20 days	(60)
	Membrane bioreactor	96±1	>98	-	>75	Flocs of membrane bioreactor has a size of 10-100 µm as to compare to 100-500 µm of conventional sludge and its floc surface per unit reactor volume is also higher	(57)
	Fixed bed reactor	90±3	>95	-	69±9	Short HRT of 35 min	(57)
Chemical/physical treatment	Pre-chlorination	33.4±9.2	19.1±16.5	28.0±7.0	23.7±5.1	Data was taken based on 100 ng L-1 concentration	(8)
	Coagulation/sedimentation	37.3±14.9	52.1±4.1	26.7±9.2	17.3±15.3	5 mg L^{-1} of alum is used as coagulant	(8)
	Rapid filtration	94.1±5.6	96.3±5.5	92.4±4.1	94.9±5.4	Crushed anthracite	(8)
	Post- chlorination	27.7±17.2	22.4±14.4	31.5±4.8	44.0±5.8	-	(8)
	Ultra filtration	90.8	98.9	40.7	98.7	Using secondary wastewater	(3)
	Cross-flow microfiltration	70	87	-	-	-	(30)

E1, estrone; E2, estradiol; E3, estriol; EE2, ethynylestradiol; CAS, conventional activated sludge; OD, oxidation ditch; WWTP, wastewater treatment plant; SRT, solid retention time; HRT, hydraulic retention time.
*Predicted value based on pseudo first order biodegradation rate coefficient normalized to biomass.

220 min.[16] Removal of estrogen is more effective with ozone compared with chlorination. Chlorination only removes 20 to 40% of the estrogenic compounds. Further steps, such as pre-chlorination and post-chlorination also did not show any noticeable difference in the overall removal rate.

Estrogen removal from water bodies is effective with activated carbon.[68-70] The activated carbons investigated for the removal of estrogenic compounds include coconut shell and wood. The amount of estrogen adsorbed by activated carbon is 25.6 to 73.5 mg g^{-1} for E1 and 21.3 to 67.6 mg g^{-1} for E2 at 1 mg L^{-1} in pure water.[69] The effectiveness of activated carbon removal was confirmed by other authors who applied activated charcoal as adsorbent to remove estrogenic compounds.[70,71] Another study suggested that estrogens in liquid manure are prone to bind with colloids with size ranging from 0.7 µm to 1.2 µm because they are the most mobile ones in porous media. The same porosity property was seen in activated carbons.

Another method used for estrogen removal is adsorption with cyclodextrin polymers. The interaction and adsorption process by cyclodextrin is the host-guest interaction through molecular recognition for the interaction of the polymer matrix. Interactions form a complex via selective incorporation into the hydrophobic cavity and nonselective incorporation into the secondary cavities of the polymer network together with hydrogen bonding for their linkages. This study showed that cyclodextrin adsorbs estrogen at a low concentration, even in the presence of other cholesterols. Although the process is effective, it merely forms complexes with cyclodextrin, and estrogens are not removed from water due to its high solubility.[14]

In Sweden, two advanced treatment plants were investigated. Apart from the conventional chemical and biological processes, namely, anaerobic and aerobic processes, an advanced treatment of slow

Table 6. Percentage removal of estrogenic compounds by different treatment methodologies in laboratory scale studies.

Method		E1%	E2%	E3%	EE2%	Additional notes	References
Biological treatment	Pure culture of iron-reducing bacteria	27	90	60	9	Duration of 15 d	(12)
	Full-functional peroxidase enzyme replicas (TAML activators)	-	-	-	<90	Preoxide catalyst is required and degradation time range from 25 min to 3 hours depending in its concentration	(67)
Chemical/physical treatment	Liquid-liquid Extraction (LLE) with decamethylcyclopentasiloxane (D5)	>99.5	90	-	>99.5	9 stages of LLE is required with ratio of D5: H_2O at 0.5:1	(13)
	β-cyclodextrin Polymer	-	70	-	-	Concentration used 10-11 mol L^{-1}	(14)
	Manganese oxide	100	100	100	90	Condition of pH 4 after 220 min	(16)
	Coagulation with Polyaluminium Chloride (PAX-18)	17	16	31	21	-	(15)
	Sorption with powdered activated carbon	100	100	100	100	-	(15)
	Sorption with Granular activated carbon	100	99.9	100	100	-	(15)
	Nanofiltration Membrane	63	78	71	90	-	(15)
	Coagulation	18	17	30	21	With aluminum sulfate	(15)

E1, estrone; E2, estradiol; E3, estriol; EE2, ethynylestradiol.

Table 7. Comparison of different treatment methodologies used for estrogenic compound removal in pilot or actual plant.

Treatment	Advantages	Limitations	References
CAS process	Usually design for large treatment capacity (~50,000 m^3 d^{-1}); 3-30 d SRT	Tendency of E1 increment after treatment	(51) (57)
OD process	Reduced discharge fluctuation with continuous discharge; less sludge produce; energy efficiency	Usually design for small treatment capacity (<5000 m^3 d^{-1}); 10-120 d SRT; high level of suspended solid in effluent; larger land area is required compare to CAS	(51) (74)
Sequential batch reactors	Equalization can be done in one reactor; flexible operation and control; hydraulic retention time (HRT) of 6-14 h (municipal load)	Periodic effluent surge; potential of discharging settled sludge during its configuration process; high level of maintenance (automated control system)	(74) (75)
Carboneous activated sludge system	Good adsorption capacity for large molecular weight compounds; smaller size of aeration tank is needed (6% of nitrifying sludge system tank)	Limited removal efficiency for small molecules; low partitioning coefficient (log K_p 0.5 for EE2); a larger amount of biomass is required (compared to nitrifying sludge system)	(59) (76)
Nitrifying sludge system	More effective than carboneous activated sludge; simpler treatment process; high partitioning coefficient (log K_p 4.3 for EE2); capability to removed and degrade estrogen compounds	Dependent on process conditions such as concentration and flow rate; temperature dependent microbial process; nutrient based activated sludge process design that requires high volume basins	(59) (77)

CAS, conventional activated sludge; SRT, solid retention time; OD, oxidation ditch; HRT, hydraulic retention time.

sand filtration was added. A study on estrogenic exposure on Juvenile rainbow trout (*Oncorhynchus mykiss*) was conducted by collecting their bile fluid. Results showed that the exposure before sand filtration was 200 times higher than the limit of quantitation. The standard value of the limit of quantitation at normal condition was 0.4 µg mL^{-1} plasma, which is the maximum quantity of estrogenic compound supposed to be detected at normal conditions in 1 mL of bile plasma. Although lower, the exposure of plasma concentration after treatment was still 20 times higher than the limit of quantitation. In terms of dry weight before the treatment, the levels of E1, 17α-E2, and 17β-E2 were 4.0, 0.25, and 0.17 µg g^{-1} compared with <0.04, <0.10, and 0.04 µg g^{-1} in the control condition.[72] Another treatment method investigated was the degradation of estrogenic compound using photolysis. However, the results obtained were not favorable because only 60% removal was recorded after 144 h.[53] Molecular imprint polymers (MIPs) have also been studied for the removal of estrogenic compounds.[73] MIPs are smart adsorbents for separation procedures and chemical analyses, possessing a high selectivity template and synthesis with physical robustness, high strength, resistance to elevated temperatures and pressures, and inertness toward organic solvents, acids, or bases.

Applications of RO treatment were also explored for estrogen removal. Virgin and fouled membranes were used for the input of estrogen concentration range between 125 to 167 ng L^{-1} and 27 to 83 ng L^{-1}, respectively. Final removal of these estrogenic compounds marked a range below 25 ng L^{-1}, giving a removal percentage of more than 80 to 85% and 8 to 70% for virgin and fouled membranes, respectively.[3]

Nitrifying activated sludge was also studied to further improve the current conventional treatment system. Ren and Nakano (2007) suggested that estrogen removal is conducted by donating electrons

Table 8. Comparison of different treatment methodologies used for estrogenic compound removal in laboratory scale.

Treatment	Advantages	Limitations	Additional notes/other applications	References
Manganese oxide	Oxidizing agent for various organic compounds including antibiotics; short treatment period (removal of 90% of 4 x 10^{-6} M of estrogen in 2 h)	Presence of metal ions (including by-product of Mn (II)) could inhibit the decomposition processes; manganese (hydr)-oxide promote polymerization of phenolic compounds	Surface area of 128 m^2 g^{-1}; application (oxytetracycline, a tetracycline antibiotics; heavy metals)	(16) (78) (79) (80)
Coagulation	Able to remove suspended solids and aid in removing dissolved organic carbon	Only able to removed <25% most EDCs; not effective in removing trace level organic pollutants	Aluminium sulphate and ferric chloride are the common used coagulants; application (municipal wastewater; algae)	(68) (81) (82)
Activated carbon	Able to effectively adsorb many organic pollutants; short contact time of 0.5-5 h	Additional process of sedimentation or filtration is required; used of activated carbon will require further regeneration or disposal	Application (dyes effluent; heavy metals)	(68) (83) (84)
Ozonation	Oxidations are effective on phenolic moieties (E1, E2 and EE2); relative low ozone doses are sufficient	Oxidation are less effective for those without phenolic moieties (progesterone and testosterone); highly selective, as removal of some compounds (*e.g*: clofibric acid and ibuprofen) requires the presence of H_2O_2; by-product of certain compound poses a serious health risk	Usually followed-by biological treatment processes; application (landfill leachates; pharmaceutical chemicals; wastewater)	(68) (85) (86) (87) (88)
Membrane filter	Able to remove microbial constituents process without increasing/reduce disinfection by-products; small space is required; able to handle a variety quality of water feed	Concentrated brine stream which need proper disposal. concentrated brine will have greater toxicity than influent water.	Application (organic matter removal; microbial removal)	(3) (89)
UV photolysis	Capable of oxidizing organic contaminants with taste and odor-causing compounds; with addition of H_2O_2 advance oxidation, it requires low fluence dose	Medium pressure lamp is required for better degradation; mineralized pollutant need extended UV treatment times; competition between EDC and H_2O_2 lead to discrepancies of energy	Application (endocrine disruptor; digestion of food samples)	(90) (91)
Peroxide enzyme	Short degradation time	May cause toxicity to fishes; oxidant and peroxide catalyst is required and its dosage is higly dependent on water quality parameter including pH and temperature	Use as catalyst in wool treatment; application (wastewater)	(92) (67)
Liquid-liquid extraction (LLE) with decamethylcyclopentasiloxane (D5)	Effective extraction of EDCs at over a short period of time; non-toxic to the environment to handle large volume;	Impractical multi-stages process required for effective removal	-	(13)

to heterotrophs or through co-metabolic degradation of ammonia-oxidizing bacteria such as *Rhodococcus zopfii and R. equi.*[61] This however contradicts with a pilot studies using seeds from several treatment plant in Washington where ammonia oxidising bacteria were found to be most unlikely to be responsible for the EE2 degradation.[60]

Comparison of treatment methodologies

All treatment methodologies come with advantages and limitations. The types of compounds to be treated, the outcomes, and the desired effluent qualities are also considered when deciding the type of treatment methods to be employed. Table 7 shows the comparison of the advantages and limitations of each treatment methodology applicable to estrogenic compounds based on pilot and actual treatment plants. Table 8 shows the comparison with laboratory-scale studies.

Majority of the studies conducted based on pilot or actual plants are usually biological systems with activated sludge, the conventional treatment system for wastewater. Several divergence and modifications have been conducted on conventional activated sludge systems such as carboneous and nitrifying activated sludge systems. The most common conventional activated sludge process is designed for large-scale usage of 50,000 m^3 d^{-1}.[51] Meanwhile, the oxidation ditch process is designed for small-capacity usage. An oxidation ditch has a more reliable performance due to its constant water level and continuous discharge. The long HRT also minimizes the impact of sudden load. However, this process requires a larger land area for its treatment process and may incur additional costs for its construction.[74] Although the nitrifying sludge system is more effective compared with carboneous activated sludge system, its performance is dependent on the processing conditions, such as concentration, temperature, and flow rate of wastewater.[59] Table 8 shows a comparison of the different types of methodologies conducted in laboratory scale for estrogenic removal. Table 6 shows that manganese oxide and activated carbon are the most effective in estrogenic compound removal. However, manganese oxide ions inhibit the decomposition process[16] and promote the polymerization of phenolic compounds.[78] Meanwhile, activated carbon requires the additional process of filtration or sedimentation, and further procedures for disposal. Coagulation, a popular methodology in color removal,[93] is the least effective method in estrogenic compound removal because it only removes approximately 25% of EDC compounds.[68]

The need for better regulation and treatment method

In most countries, the amount of estrogenic compounds is not a parameter that is used to determine the effectiveness of a wastewater treatment plant. Hence, plant operators do not check the levels of estrogen in their effluents. Better regulations which include a new parameter, *i.e.* the levels of estrogenic compounds in the effluent should be introduced in order for people in general to understand the importance of limiting these compounds into our water bodies. Fortunately, the detrimental effects of estrogenic pollution has drawn the attention of the European Union Commission whereby estrogenic compounds has been added to the monitoring data watch list under article 8b in its revision.[94]

The efficiency of sewage treatment plants on estrogenic compound removal is in the range of 50 to 95%. The most common treatment method used in removing estrogenic compounds is the biological method, which usually employs the activated sludge system. Although activated sludge removes almost 100% of estrogenic compounds, the retention time required is more than 10 d. The adsorption capacity of activated sludge is dependent on temperature, sludge age, hydraulic retention time, sewage composition, and its dilution. With the long duration of retention time required for favorable removal, employing this system is not cost-effective because a huge retention pond needs to be built. A combination of several methodologies, such as the use of manganese oxide, activated carbon, and biological treatment, should be considered. A combination of these systems may provide an almost complete removal of estrogenic compounds. This integrated method is expected to be sustainable and can produce harmless by-products and remove estrogens at a relatively short retention time.

Conclusions

Exposure to pollutant in the form of excessive estrogenic compounds discharged into water bodies has caused various detrimental effects on fish and other aquatic organisms. Various methods of removal, along with their advantages and disadvantages, have been reviewed and discussed. However, estrogenic pollution still occurs worldwide; thus, an environmental regulation that includes estrogenic compounds as one of their parameters should be introduced. In addition, more research should be conducted to determine a more effective and efficient treatment system that would require less retention time. Further research is also required to investigate and develop a simple estrogen determination method that can be standardized for the use of all researchers and practitioners worldwide.

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