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REMOVAL OF STEROID HORMONES AND PERSONAL CARE PRODUCTS IN WASTEWATER BY CHEMICAL PRECIPITATION

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ABSTRACT

The presence of steroid hormones and endocrine disrupting compounds (EDC) in the environment has been connected with the drop in semen quality in men and the number of hermaphrodite fish observed downstream wastewater treatment plants. EDC originating from down-the-drain chemicals can be reduced by mitigation options but the naturally occurring hormones must be removed though end-of-pipe treatment. In this study, coagulation and flocculation as well as these two techniques combined with sorption were applied to remove estrone, 17β-estradiol and the synthetic hormone 17α-ethynylestradiol as well as the preservatives methyl paraben, ethyl paraben, propyl paraben, butyl paraben and isobutyl paraben from primary and secondary treated municipal wastewater. It was found that coagulation with both iron and aluminium together with an anionic flocculant successfully removed organic matter and dissolved phosphorous but not the hormones and only up to 30% of the parabens. This was seen both in the chemical analyses of the individual substances and well as in an assay of the oestrogenic effects. Applications of powdered activated carbon pre- and post the chemical coagulation-flocculation significantly increased the oestrogen removal, which is consistent with existing literature. The treatment processes in the studied wastewater treatment plant removed both the oestrogens and the parabens to below the limit of detection, though a detectable but small oestrogenicity in the effluent cannot be disregarded.

KEYWORDS

Coagulation; Estrogens; Flocculation; Oestrogens; Parabens; Powdered activated carbon; Sorption, Xenobiotic organic compounds; YES assay.

1 INTRODUCTION

The presence of steroid hormones and endocrine disrupting compounds (EDC) in the environment has received a growing attention since the 1990´s. The connection between exposure to oestrogen hormones and other organic chemicals and the drop in semen quality seen in men has been put forward by Sharp and Skakkebæk [1]. Hormones and EDC does not only affect humans but also other organisms and oestrogenic effects have been observed, for example, in rainbow trout in wastewater affected recipients [2,3]. These effects are caused by the exposure from excreted natural hormones and synthetic oestrogens from contraceptive

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pills [4] but also from down-the-drain chemicals such as phthalates and parabens which are also suspected of affecting the hormone system [5,6]. Surface waters have been found to contain oestrogens [7-10] in levels that exceed the concentrations that results in oestrogenic effects in fish [2,6], thus adducing that effects in the aquatic ecosystem are to be expected. Furthermore, in influents to wastewater treatment plants even higher concentrations have been found either through chemical analysis or calculations of E2 equivalents based on YES assays [11-15] demonstrating that there is a substantial need for treating wastewater with respect to the steroid hormones. Similarly, the parabens, a group of biocides among other things used in personal care products, have been found in surface waters [9,16] and in wastewater and grey wastewater [14,16,17]. This does however not exceed the effect concentrations seen in fish [5,18] but measurements are scarce and the degree of additive effects have not been investigated.

There are two major options for reducing the presence of unwanted substances in wastewater; upstream source control and end-of-pipe treatment. As the steroid hormones are naturally excreted from the human body they will be inherently present in wastewater and thus treatment will be required. For the parabens there are however possibilities to mitigate their presence, e.g., substituting with other biocides, which is currently occurring in Denmark as butyl paraben has been phased-out in leading cosmetic brands [19]. In wastewater treatment, chemical precipitation with iron or aluminium salts does not reduce the oestrogenicity whereas lime precipitation is more effective [21]. Chemical treatment is primarily used for phosphorus removal but organic and suspended matter is also removed simultaneously [20], thus not specifically targeting hormones and EDC. The information regarding removal of hormones and EDC by coagulation and flocculation using multivalent cations in combination with organic flocculants is sparse if non-existing.

The aim of this study is to test the hypothesis that chemical coagulation in combination with organic flocculants and/or a sorbent can remove steroid hormones and selected down-the-drain chemicals as well as the observed oestrogenic effects from wastewater.

2 MATERIALS AND METHODS

2.1 Physico-chemical properties

The compounds included in this study were the natural hormones estrone (E1), 17β-estradiol (E2) and the synthetic hormone 17α-ethynylestradiol (EE2) as well as the preservatives methyl paraben (MP), ethyl paraben (EP), propyl paraben (PP), butyl paraben (BP) and isobutyl paraben (IBP). As can be seen in *Table 1*, the hormones are neither easily water soluble nor volatile but prone to sorption based on the octanol-water partition coefficient (Kow) and the organic carbon partition coefficient (K_{oc}). The oestrogens are excreted from the body as the more hydrophilic glucuronides or sulphates complexes but deconjugation of the glucuronides primarily takes place in the sewer system [21]. The parabens are highly water soluble and less likely to sorb onto solid phase, though the sorption is likely to increase with increased chain length, *Table 1*. They are excreted from the body as para-hydroxybenzoic acid or conjugates thereof [22]. Therefore parabens in the wastewater originates from personal care products rinsed off during use, for example, shampoos and shower creams.

Table 1. Physico-chemical properties of the hormones and parabens considered in this study.

Name	E2	EE2	E1	MP	EP	PP	BP	IBP
CAS no.	50282	57636	53167	99763	120478	94133	94268	4247023
Sw (mg/l)	3.6[1]	11.3[1]	30[1]	2496[1]	885[1]	463[1]	250[1]	223[3]
pKa	10.71[6]	10.44[2]	10.77[6]	7.91[2]	8.34[1]	8.23±0.2[2]	8.5[3]	7.91[2]
LogKow	4.01[1]	3.67[1]	3.13[1]	2[3]	2.47[1]	3[1]	3.5-3.6[1]	3.4[3]
Koc	4205[5]	4678[5]	4477[5]	126[1]	119-209[1]	879[1]	788[3]	660[3]

1) [23] 2) [24] 3) [25] 4) [26] 5) [27] 6) [28]

2.2 Treatment processes

Coagulants are chemicals typically multivalent cations that contribute to molecular aggregation as dissolved substances are aggregated into microscopic particles. These particles may be flocculated into macroscopic floc with assistance of a flocculant. Flocculants can be long-chain polymers of modified polyacrylamides which are chemicals that promote flocculation by aggregating colloids and other suspended particles into flocs, thus, allowing removal by filtration or sedimentation [29]. The adsorption process is the action of attaching one substance to the surface of another substance or matter. It is used here for removal of a pollutant from wastewater by collecting it on the surface of a solid material, e.g., activated carbon. Combination of coagulation and sorption has shown that the joint process can decrease the presence of organic matter measured as chemical oxygen demand (COD) beyond that of the individual treatment steps [30].

2.3 Jar-test setup and related chemicals

Test of chemical coagulation with metals was assessed using a standard jar-test with four to six 1-L glass jars. One minute of rapid stirring (400 rpm) were used to disperse the coagulants. Coagulation was carried out via 15 minutes of slow stirring (50 rpm) and finally the jars were allowed to stand for 30 minutes for gravitational settling of flocs. Test of coagulation-flocculation with metals and polymers was assessed by 1 minute of rapid stirring, 50 rpm at 15 min followed by addition of the polymer during rapid stirring at 400 rpm during 20 seconds followed by settling in 30 minutes. In tests with the sorbent, a pulverized activated carbon (PAC), in combination with coagulation-flocculation, the PAC was either added before the coagulant or after the flocculant. The coagulants used were polyaluminium chloride (PAX-XL-100) and iron (III) sulphate (PIX-111) both were supplied by Kemira Kemwater, Sweden). Polymers (anionic (Superfloc A-130 and Fennopol A-392) non-ionic (Superfloc N-300) and cationic (Superfloc C-496)) were also supplied by Kemira (Kemira Miljø A/S, Denmark). The sorbent used was pulverized activated carbon (<45 μm (90%); Flemming Zwicky ApS, Denmark).

2.4 Wastewater samples

The tested wastewaters used in this study were collected at Avedøre Wastewater Services (Avedøre Spildevandscenter I/S), which is an urban wastewater treatment plant located in the western suburbs of Copenhagen, Denmark. The load of organic matter corresponds to 325,000 person equivalents (PE) and about 70% of the wastewater is domestic wastewater, about 30% is of industrial origin and from two large hospitals. The treatment process contains mechanical treatment, biological treatment via nitrification/denitrification in an activated sludge process as well as chemical phosphorous removal by post-coagulation with JKL (Iron(III)chloride sulphate) supplied by Kemira. Preliminary treated wastewater (after 10 mm screening, sand /grit removal) and secondary treated wastewater (after biological treatment before addition of coagulant) were used for the coagulation-flocculation experiments. Samples were spiked with hormones (1 and 5 µg/L of the individual compounds) and parabens (2.5 and 12.5 µg/L of the individual compounds).Two flow proportional day average samples from the influent and effluent were collected in order to assess background concentrations in the treatment plant.

2.5 Chemical analyses

Total organic carbon (TOC) was measured using high-temperature combustion (Shimadzu TOC 5000A). Samples were taken ca. 2 cm below the surface of the jars and subsequently frozen up to analysis and measured within two weeks from sampling. The limit of detection was 0.9 mg/L and the linear range 1 to 50 mg/L. Chemical oxygen demand (COD) was measured according to the Danish Standard Method (DS 217:1991). Similar to TOC, COD was sampled below the surface and frozen prior to analysis. The limit of detection was 5 mg/L and the linear range 50 to 600 mg/L. Phosphate was measured using a spectrophotometric method (Spectroquant® Photometer NOVA 60; ortho-phosphate 14848 from MERCK). The analyses were carried out immediately after sampling or directly frozen up to analysis. The limit of detection was 0.05 mg/l and the linear range 0.05 to 4.0 mg/L. The hormones and parabens were analyzed according a revised version of a method for analysis of oestrogens by Ternes and colleagues [10]. Limit of detection (LOD) for E1, E2 and EE2 were 10, 27 and 10 ng/L, respectively. For the parabens the LODs were MP 160, EP 26, PP 32, IBP 21, and BP 62 ng/L. The linear ranges were 10 to 200 and 10 to 500 ng/L, for the hormones and parabens, respectively. The recovery was found to be 80, 40 and 50% for E1, E2 and EE2.

2.6 Yeast oestrogen-screen (YES) assay

The YES assays were performed according to Routledge and Sumpter [31]. The samples were preserved by buffering to pH 3. They were then concentrated by SPE with a C18 column (IST) and eluated with acetone after which the eluate was evaporated to dryness and re-dissolved in 300 mL ethanol. A number of dilutions were made after which the yeast culture was added. The responses, absorbance of coloured complexes, were measured at 540 and 630 nm with a Hitachi F2000 Fluorescence Spectrophotometer at the start and after 3 days of incubation. The results were compensated for the background absorbance seen in the wastewater samples.

2.7 Statistical analysis of variance

In order to test if any of the treatments varied from one another (zero-hypothesis means that all treatment are the same), an analysis of variance (ANOVA) was performed using 95% statistical significance. In the cases where the zero-hypothesis was rejected (e.g. some of the treatments differed significantly), Tukey's test were used [32] using 95% statistical significance in order to establish which of the treatment combinations of coagulants, flocculants and sorbent that differed statistically from each other and the controls.

3 RESULTS AND DISCUSSION

3.1 Optimization of test parameters

An optimization of the metals (two or four levels were tested; 4.8, 9.6, 20 and 39 mg Fe/L as well as 6.5 and 19 mg Al/L) showed that an increased removal of TOC could be seen with increasing coagulant dosage.

Table 2. Removal of organic matter by coagulation-flocculation

	A (Superfloc)	A (Fennopol)	N	C
No coagulant	+		-	+
Al	++		-	+
Fe	+++	+++	-	+

- no removal; + <30% removal; ++ >30% removal; +++ >50% removal

The difference was not substantial between the lowest and the middle concentration but apparently different to the highest concentrations. Similarly for the polymers (two levels were tested for each polymer; 6 and 12 mg/L), respectively) it was shown that the use of the anionic polymers and to a lesser extent the cationic polymer improved the removal of TOC compared to usage of the metal coagulants alone. But the use of the non-ionic polymer showed no such effect, *Table 2*, and instead sometimes an increase in the TOC concentration could be seen. No difference in removal efficiencies could be seen when comparing the two anionic polymers with each other (based on COD removal). The remaining tests were carried out using the highest metal levels and the lowest concentration of anionic polymer. All tests included a sample of untreated wastewater for comparisons. Additionally, three levels of PAC (10, 50 and 250 mg/L) were tested on both pre- and post treatment, i.e., prior to coagulant or jointly with the flocculants, with a contact time of 15 or 60 minutes. Combination of coagulation and sorption has shown that the joint process can decrease the presence of organic matter measured as COD beyond the individual treatment steps. 100 mg polyaluminium chloride/l removed 43% of the COD from the wastewater and likewise did 50 mg/l PAC when applied individually. The combined process removed 57% [30]. The coagulation-flocculation removed less organic matter than the whole treatment train at Avedøre but achieved a high orto-phosphate removal, *Table 3*.

Table 3. Removal of organic matter and phosphate in this study and at Avedøre WWTP.

Percent removal (%)	Chemical coagulation and flocculation (Fe+A)	Chemical coagulation and flocculation (Al+A)	Chemical coagulation-flocculation (Fe+A) and sorption	The whole treatment plant [13]
TOC	56	26	-	
COD	61	-	59-64	95
PO_4-P	>97	96	94-99	92 (P_{tot})

3.2. Removal of steroid hormones

The coagulation-flocculation tests were performed in triplicates but due to the intra-sample variation (using ANOVA) no distinct variance between the hormone concentrations in the samples could be seen, see *Figure 1*. Coagulants have been found to remove E1, E2 and EE2 in artificial surface waters yielding a 17-22% removal using polyaluminium chloride and 4-17% using ferric sulfate [34]. The polyaluminium chloride has also been used for treatment of real and artificial surface waters producing an E1 removal of 5% [35], E2 removal of <5% [36] and 2% as well as an insignificant EE2 removal [38]. Chang and colleagues [37] found that ferric chloride did not consistently remove E1 to any significant extent from the secondary treated wastewater. Avedøre influent and effluent concentrations: influent 56 and 97 ng/L of E1, all remaining samples were below the limit of detection. Previously the concentration of E1, E2 and EE2 has been measured in three samples at Avedøre [13] to range

19-75, 6.1-27 and <1-1.7 ng/l in the influent and 5-11, <1-4.5 and <1-5.2 in the effluent. This corresponds with an E1 removal of 42-93 % which correlates with what was observed in this study (>82 and >90%).

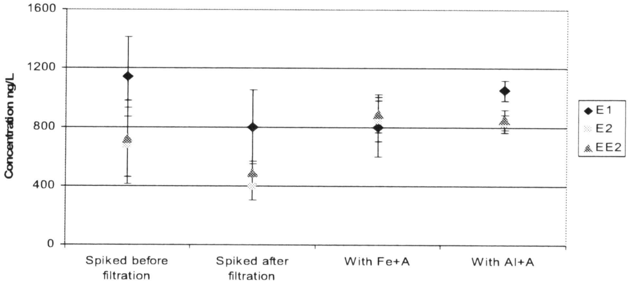

Figure 1. Concentrations of steroid hormones found in the jar-test (triplicates).

3.3. Removal of parabens

Parabens were spiked and analyzed in the same manner as described for the hormones. The smaller (short-chain) parabens were not removed by chemical coagulation-flocculation but the longer (4 carbon atom chains) BP and IBP were to a limited extent (about 30%). The background concentrations in the treatment plant, measured in the influent, ranged from MP 930-1500 ng/L, EP 330-670, PP 920-1200, BP 240-260 and IBP 100-110 ng/L. The levels of parabens in the effluent were all below the limits of detection. When the concentration has fallen below the limit of detection no precise value can be given and thus, the minimal removal (down to the LOD) is >83% for MP, >92% for EP, >97% for PP, >91% for BP and >38% for IBP.

3.4. Removal of oestrogenic effects

The YES response of the spiked dissolved non-conjugated hormones and the parabens were measured and concentration-response curves were obtained for the different treatment test samples as presented in *Figures 2 to 5*. Clear concentration-response relationships were found for all samples. A statistical analysis (ANOVA) of the three replicated from each tested treatment (A, Fe+A and Al+A) did not (with a 95% certainty) show differences between the treatments, and thus, the YES assay support the findings in the chemical analyses that the combination of coagulants and flocculants does not remove the oestrogens and hence oestrogenic effects.

In addition to the noted oestrogen effects seen in the influent samples into Avedøre and the spiked control, toxic effects could be noted. In the effluent a small but detectable effect could be seen, corresponding to an E2-equivalent of 0.7 ng /L, but since the value is low compared to the standards it is associated with a great deal of uncertainty, *Figure 2*. Addition of the sorbent, PAC, did however yield a significant removal of the oestrogenic effects, see *Table 4*, with a logarithmic relationship between the oestrogen removal and the PAC concentration. There was not statistical difference in treatment efficiency between the pre- and post addition of the sorbent and neither in the 15 or 60 minutes contact time, not shown.

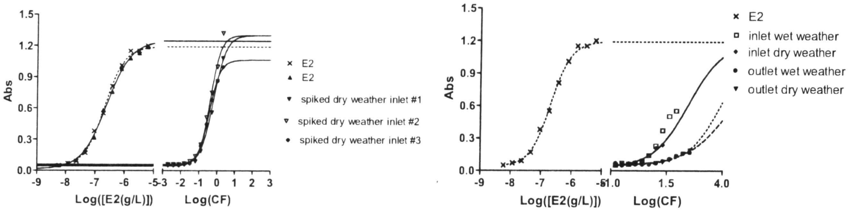

Figure 2. YES response to spiked influent samples (left) and unaltered in- and effluent samples.

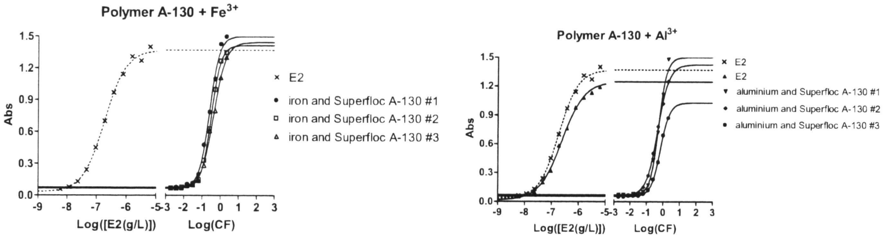

Figure 3. YES response to treatment with anionic polymer and metallic coagulant (iron, left and aluminium, right).

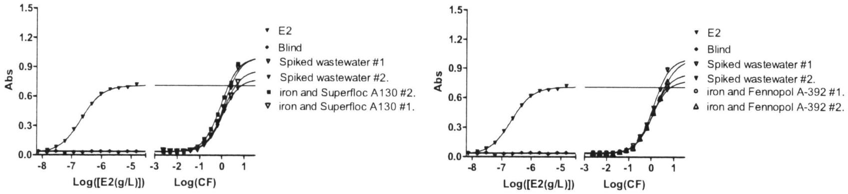

Figure 4. YES response to treatment with iron coagulant and the two anionic polymers (Superfloc A-130, left and Fennopol A-392, right).

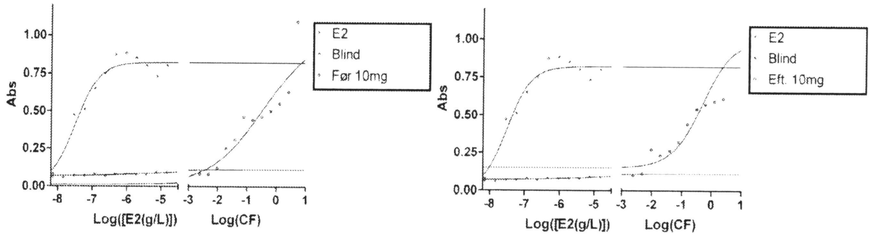

Figure 5. YES response to treatment with iron coagulant, anionic polymer and PAC sorbent where the sorbent was added before (left) and after (right) the coagulant.

This is comparable with literature, as application of PAC treatment to surface water resulted in a removal of 76% E1, 84-94% of E2 and 74-88% of EE2 [35]. Correspondingly, in raw drinking water a removal of 87->99% E2 and 50 - >99% EE2 were obtained after 4 hours contact time [38]. Over 90% was removed regardless contact times, though generally longer contact times and higher PAC doses lead to higher removal [40]. Chang and colleges [37] used the same approach for secondary treated wastewater attaining a maximum estrone removal of 95–96% where the E1 concentration decreased with increase in PAC dosage.

3.5. Comparison of the chemical analyses and the bioassays

In order to compare the response seen in the YES assay with the chemical analyses the concentrations of E1, E2 and EE2 were calculated into E2-equivalents, see *Table 4.* The parabens were omitted here due to the minute contribution to the oestrogenicity compared to the oestrogens. E1 has a relative E2 potency of 0.2 [33] and EE2 a minimum of 1 [2] and hence the minimum relative potency (RP) is calculated according to *Equation 1.*

Equation 1: $\mathrm{RP} = 0.2 \times C_{E1} + C_{E2} + C_{EE2}$

Table 4. Relative potency (RP) based on the measured concentrations of E1, E2 and EE2 and from the YES assay response in estradiol equivalents (EEQ)

Spiked concentrations (individual concentration of E1, E2 and EE2)	1000 ng/L n=3		1000 ng/L n=2		5000 ng/L n=3	
	EEQ	RP	EEQ		EEQ	RP
			pre	post		
A (Superfloc)	439	1980			1338	1209
Fe_{39}+A (Superfloc)	604	1884			4688	6310
Al_{19}+A (Superfloc)	364	1876			1905	5788
Fe_{20}+A (Superfloc)			275			
Fe_{20}+A (Fennopol)			421 (n=1)			
$Fe_{9.6}$+A (Superfloc)			205			
$Fe_{9.6}$+A (Fennopol)			147			
Fe_{20}+A (Superfloc) PAC_{10}			107	67		
Fe_{20}+A (Fennopol) PAC_{50}			24	20		
Fe_{20}+A (Superfloc) PAC_{250}			14	85		
Control	553	1617	178		4335	4940

Control = spiked primary wastewater; the concentrations of the anionic flocculants were in all tests 6 mg/L

For the YES assays estradiol equivalents (EEQ) were obtained via calculations in the GraphPadPrism software, which also was used to draw *Figures 2-5*. In general the YES assay had a lower response then the RP calculated for the chemical analyses. This may be a drawback from not including an internal standard in the SPE procedure which may have resulted in a loss of reactive substances prior to the YES assay. However, in some cases, as shown in *Figure 2*, toxic effects seen in the YES assay prevented a full dose-response curve.

4 CONCLUSIONS

Coagulation alone is known not significantly to remove steroid hormones and oestrogenicity from surface and drinking water and in this study it was shown that coagulation in combination with using these specific organic flocculants is not suited as the single removal mechanism for steroid hormones in wastewater. Though it was proven to significantly remove both organic matter and dissolved phosphorous. Coagulation-flocculation in combination with powdered activated carbon does however remove the majority of the oestrogen effect. The existing treatment processes in Avedøre WWTP was found to remove both the parabens and the estrogens to below the LODs. Oestrogenicity in the effluent water cannot be excluded as indications effects could be see in the YES assay.

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