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Degradation and estrogenic activity removal of 17β-estradiol and 17α-ethinylestradiol by ozonation and O_3/H_2O_2

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ABSTRACT

This work investigated the degradation of a natural (17β-estradiol) and a synthetic (17α-ethinylestradiol) estrogens (pure or in the mixture) and the removal of estrogenic activity by the ozonation and O_3/H_2O_2 process in three different pHs (3, 7 and 11). The effect of oxidation via OH radical was evaluated adding a radical scavenger (t-butanol) in the medium. Estrogenic activity was performed using the YES assay. 17β-estradiol and 17α-ethinylestradiol presented similar estrogenic potential and the association of these estrogens resulted in an addictive effect for estrogenic activity. Ozonation and O_3/H_2O_2 processes were effective in removing the estrogens in aqueous solution. In the mixture at pH 11, removals were higher than 98% and 96% for 17β-estradiol and 17α-ethinylestradiol, respectively. In pH 3, 17β-estradiol and 17α-ethinylestradiol removals were 100% and 99.7%, respectively. When estrogens were treated separately, the removals in pH 11 were superior to 99.7 and 98.8%, while in pH 3 were 100% and 99.5% for 17β-estradiol and 17α-ethinylestradiol, respectively. 17α-ethinylestradiol has been always removed at lower rates (pure or in the mixture) for all applied conditions. Estrogenic activity was completely removed in pH 3 for ozonation or O_3/H_2O_2. The samples oxidized in pH 11 presented higher estrogenic activity than those in pH 7. Estrogens removal was lower at pHs 7 and 11, when the scavenger was added to the media. The higher estrogen residual concentrations found in ozonation in presence of tert-butanol are contributing for higher estrogenic activity observed in pHs 7 and 11. By-products with estrogenic activity were formed by oxidation via OH radical. Only a few compounds could be identified in pHs 7 and 11 and they have a phenolic ring, which, probably is contributing to the estrogenic activity observed.

1. Introduction

There is a growing concern about water quality all over the world. Recently, many studies reported alterations on the reproduction of animals and humans. They are possibly associated to the presence of some micropollutants, known as "Endocrine Disrupters Chemicals" (EDCs), found in superficial and underground waters. Works published in the literature showed that endocrine disrupters can increase the incidence of cancer of the testicle, ovary and breast as well as to reduce fertility and spermatozoids number and promote fish feminization (Coleman et al., 2005; Harrison et al., 1997).

Natural and synthetic estrogens are the main substances responsible for estrogenic activity found in domestic sewage. Estrogens 17β-estradiol and 17α-ethinylestradiol are excreted daily by humans in domestic sewage and are only partially removed in domestic wastewater treatment plants (DWTP). Consequently, they are continuously discharged into receiving bodies (Ternes et al., 1999). 17β-estradiol is the main natural estrogen responsible by feminine characteristics

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formation. 17α-ethinylestradiol is a synthetic estrogen found in contraceptive pills and applied in hormonal reposition therapies.

Natural and synthetic estrogens are effective endocrine disrupters at ng l^{-1} levels (Nogueira, 2003; Routledge et al., 1998). They are among the substances which cause endocrine alterations on aquatic organisms (Gomes et al., 2004; Lai et al., 2002). *In vivo* potency of 17α-ethinylestradiol is 10–50 fold higher than that of 17β-estradiol, probably due its lower metabolization (Brian et al., 2005). Already 0.1 ng l^{-1} of 17α-ethinylestradiol induces the expression of vitellogenin in fish, 0.1–15 ng l^{-1} can affect sex differentiation and 2–10 ng l^{-1} may affect fecundity. Life-long exposure to 5 ng l^{-1} leads to significant reduction on fish fecundity (Fent et al., 2006; Nash et al., 2004). Thus, given that its concentration in the environment frequently is between 0.5 and 7 ng l^{-1} (Bila and Dezotti, 2003), 17α-ethinylestradiol may be a significant contributor to reproductive disfunction in wild fish (Fent et al., 2006).

Estrogenic activity of a substance is defined as its capacity to bind to the estrogen receptor and elucidate an estrogenic response. In this context, the molecular structure of the pollutant is very relevant. The presence of a polar group capable to form hydrogen bridge (hydroxyl, for example) with an aromatic ring seems to be of paramount importance to an estrogenic response (Hamblen et al., 2003). Studies suggest that there is only one perfect bind of 17β-estradiol molecule with estrogen receptor; i.e., through the phenolic ring of the steroid.

There are a variety of *in vivo* and *in vitro* assays to evaluate estrogenic activity. *In vivo* assays use parameters as sexual organs weight, cell differentiation, expression of proteins and enzymatic activity (Gray Jr. et al., 1997; Baker, 2001). *In vivo* experiments for investigating estrogenic effects are, in general, time-consuming and expensive. However, several *in vitro* assays have been established to identify estrogenic potentials in environmental samples. Among *in vitro* assays there are those that evaluate the interaction with hormonal receptors and cell proliferation, such as YES and MCF-7 assays, respectively.

The presence of endocrine disruptors in sewage treatment plants and sources of potable water indicates the need for evaluating the treatment processes and their efficiency in removing such substances. Ozonation, photocatalysis, O_3/H_2O_2 and O_3/UV, showed to be promising techniques for removing these micropollutants (Bila et al., 2007; Ternes et al., 2003; Huber et al., 2003; Zwiener and Frimmel, 2000; Irmak et al., 2005). According to Bila et al. (2007) ozonation was very efficient for removal of 17β-estradiol in aqueous solutions (>99%), using low ozone dosages. According to Ternes et al. (2003), for ozone doses of 15 mg l^{-1}, the natural estrogen estrone was reduced below the limit of quantification. Irmak et al. (2005) showed that the time needed for complete conversion of 0.1 mmol of 17β-estradiol was 55 min for the applied O_3 dose of $15.78 \cdot 10^{-3}$ mmol min^{-1}. According to Huber et al. (2003) 17-estradiol and 17α-ethinylestradiol exhibited high rate constants with ozone, showing that ozonation and advanced oxidative processes are promising processes for efficient removal of pharmaceuticals in waters. However, in addition to the removal of the endocrine disrupter it should be assured that the biological activity is also removed, since some reactions products may be active.

The objective of this study was to evaluate the effect of molecular ozone and hydroxyl radical for removing estrogenic activity during degradation of 17β-estradiol, 17α-ethinylestradiol and their mixture by ozonation and O_3/H_2O_2 process. The influence of pH and ozone dosage in the removal of these substances and in the formation of by-products was investigated.

2. Materials and methods

2.1. Chemicals

17β-Estradiol (98% purity), 17α-ethinylestradiol (98% purity), BSTFA (bis(trimethylsilyl)tri-fluoroacetamide), KH_2PO_4, $(NH_4)_2SO_4$, $MgSO_4$, $Fe_2(SO_4)_3$, L-leucine, L-histidine, adenine, L-arginine-HCl, L-methionine, L-tyrosine, L-isoleucine, L-lysine-HCl, L-phenylalanine, L-glutamic acid, L-valine, L-serine, thiamine, pyridoxine, calcium pantetonate, inositol, D-glucose, aspartic acid, L-threonine, copper sulfate (II) and KOH pellets were supplied by Sigma-Aldrich. Biotin and absolute ethanol were supplied by Merck. Hexane, methanol, acetone and tert-butanol were supplied by Tedia Brazil. Chlorophenol red-β-D-galactopyranoside (CPRG) was supplied by Roche Diagnostics GmbH.

2.2. Stock solutions

Stock solutions of 17β-estradiol and 17α-ethinylestradiol were prepared at 100 mg l^{-1} in acetone and stored at 4 °C. Samples for ozonation and O_3/H_2O_2 were prepared by spiking Milli-Q Biocell water with the stock solution in order to achieve initial concentrations of 10 μg l^{-1} and 50 μg l^{-1} for each estrogen.

2.3. Experimental set-up

Experiments were carried out in an ozonation unit. This unit is constituted of an ozone generator (Unitek—model UTK), a glass contact column (500 mm height × 70 mm diameter – 1.0 l volume) and an ozone analyzer for the gas phase (IN USA, ASX-Mod H1). The ozone generator is able to produce up to 5 g O_3 h^{-1}, using a blend of pure oxygen and pure nitrogen as the feed gases at a flow rate of 93.6 g h^{-1} (1.56 g min^{-1}). This mixture was used instead of air in order to allow operation with low ozone concentrations.

Ozonation experiments were performed in aqueous solution at different initial pH values: 3, 7 and 11. Sulfuric acid or sodium hydroxide was used to adjust the solution pH. Tests to evaluate degradation and estrogenic activity were performed using initial concentrations of 10 μg l^{-1} for each estrogen separately and 20 μg l^{-1} for the mixture (10 μg l^{-1} of each estrogen). For UV spectrophotometry, an initial concentration of 50 μg l^{-1} for each estrogen was used. The ozone consumed concentration ranged from 1.0 to 25 mg O_3 l^{-1}, corresponding to ozonation times of 10 s to 7 min. The ozone consumed concentration corresponds to the amount of ozone absorbed by the aqueous solution volume and was calculated using the ozone gas concentration at the inlet and the outlet of the bubble column, the liquid volume and the ozonation time.

To identify the by-products formed during ozonation, aqueous solutions containing 1 mg l^{-1} of 17β-estradiol and