Application of a yeast-based assay protocol developed to monitor total oestrogenic activity induced by 17β-oestradiol in activated sludge supernatants from batch experiments

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Abstract

Batch experiments were carried out with activated sludge from laboratory reactors and a full-scale treatment plant spiked with 17β-oestradiol (E2). An oestrogen-sensitive yeast-based assay protocol, described in detail in a related publication, was used to measure reduction of E2-induced total oestrogenic activity from the sludge supernatant over a 15 d period after which the sludge was re-spiked to check for possible enhancement of reduction by pre-exposed sludge during an additional 15 d period. The reduction was generally improved by increasing sludge solids concentrations and by continuous mixing. For a 100 ngE2/ℓ spike there was >40% reduction of oestrogenic activity within 15 d, which improved to >70% by pre-exposing the sludge. The oestrogenic activity produced by a dose of 100 μgE2/ℓ was readily removed by most sludges within 15 d. However, re-spiking the activated sludge with the same E2 concentration caused some sludges to lose reduction capacity.

Keywords: activated sludge, 17β-oestradiol, oestrogen, oestrogen receptor, oestrogenic activity, suspended solids, wastewater treatment, yeast assay

Introduction

Environmental oestrogens are environmental contaminants that can mimic the biological activities of the female hormone oestrogen in the endocrine system, i.e. they act as endocrine disrupters. Several substances are reported to have oestrogen-like activity. These include steroid hormones, synthetic oestrogens (xeno-oestrogens), environmental pollutants and phyto-oestrogens (plant oestrogens) (Arnold et al., 1996; Routledge and Sumpter, 1996; Coldham et al., 1997; Hu et al., 2000; Körner et al., 2000).

Some of these environmental oestrogens share physical and chemical characteristics which make them able to behave like, or induce a hormonal response similar to 17β-oestradiol (E2). These characteristics include their chemical structure, water solubility, and affinity for organic matter. Others do not share any structural resemblance, but can still induce a similar biological response (Zacharewski, 1997). This paper limits the definition of environmental oestrogens to those substances that can bind to and activate the oestrogen receptor (ER), and will refer to them as oestrogenic compounds or oestrogens. Primary emphasis is placed on the steroid oestrogen E2 found in domestic wastewater treatment.

Estrogens in wastewater treatment plants

Where there is a conventional wastewater collection and treatment system, some oestrogens will eventually pass through a wastewater treatment plant before being discharged to the environment (Ternes et al., 1999b). The oestrogenic input to rivers by treatment plant effluent can be quite significant. Körner et al. (2000) found E2-equivalent concentrations of between 2.5 and 25 ng/ℓ in effluent from municipal wastewater plants in southern Germany. Effluents may have up to 50 ng/ℓ of E2 alone (Desbrow et al., 1998; Belfroid et al., 1999; Layton et al., 2000) and total oestrogenic activity up to 150 ngE2-equivalent/ℓ (Körner et al., 2000; Tilton et al., 2002; Pawlowski et al., 2003; Pawlowski et al., 2004). Typical effluents have less than 20 ngE2/ℓ. While many receiving water studies have been carried out on river and lake water, there is now concern that ocean effluent outfalls may be a source of oestrogens to coastal marine environments (Atkinson et al., 2003). Solids disposal may also be a source of oestrogen to the environment from treatment plants as the hydrophobic oestrogens are expected to associate with the organic solids in treatment processes (Johnson et al., 1999). Sludge E2 concentrations of up to 4.3 ng/ℓ were measured by Murk et al. (2002).

Oestrogen reduction and organic solids in wastewater treatment

Steroid oestrogens have low water solubility – generally in the low mg/ℓ range (Tabak et al., 1981; *Merck Index*, 1996) – but at typical ng/ℓ concentrations found in wastewaters they will be in solution in samples to be analysed. However, some quantities of oestrogens will also be associated with solids and must be accounted for. Domestic wastewater typically has between 80 to 290 mg/ℓ of total organic carbon (TOC) and some treatment processes like the activated sludge (AS) system have up to 10 g/ℓ of organic solids (Tchobanoglous et al., 1991). Steroid oestrogens have relatively high K_{ow} values with calculated and experimental values of log K_{ow} reported in a range of 2.45 to 4.15 (Hansch et al., 1995; Hu et al., 2000; Lai et al., 2000). Their implied hydrophobicity means that some of the dissolved oestrogenic compounds may partition out of the water phase onto the

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