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

E Wozei* and SW Hermanowicz

Department of Civil and Environmental Engineering, 629 Davis Hall, University of California at Berkeley, Berkeley, CA, 94720

Abstract

A yeast-based assay protocol developed for detecting oestrogenic activity in activated sludge (AS) supernatant is described. The protocol used *Saccharomyces cerevisiae* construct RMY/ER-ERE with human oestrogen receptor (ERα) and *lacZ* reporter genes, and was developed by modifying existing assays for use with AS samples from batch experiments. The method was able to detect total oestrogenic activity (without prior extraction) in supernatants of AS spiked with 17β-oestradiol (E2) with a detection limit of 0.03 ngE2-equivalent/ℓ and an overall quantification limit of 100 ngE2-equivalent/ℓ. Mean E2-induced oestrogenic activity recoveries of >56% were obtained from the spiked samples.

Keywords: activated sludge, oestradiol, oestrogenic activity, suspended solids, *Saccharomyces cerevisiae*, yeast assay

Introduction

Environmental oestrogens are contaminants that can mimic the biological activities of the female hormone oestrogen in the endocrine system, acting as endocrine disrupters. Several substances are reported to have oestrogen-like activity including steroid hormones, synthetic oestrogens (xenoestrogens), environmental pollutants and phyto-oestrogens (plant oestrogens) (Arnold et al., 1996; Routledge and Sumpter, 1996; Coldham et al., 1997; Körner et al., 2000; Miyamoto et al., 2003). Effects on the reproductive system due to exposure to environmental oestrogens have been observed including endocrine disruption in fish (Harries et al., 1997; Harries et al., 1999; Christiansen et al., 2002) and other potential effects include male reproductive disorders (Ganmaa et al., 2001).

Natural steroid oestrogens like 17β-oestradiol (E2), oestrone (E1) and oestriol (E3) are produced by the mammalian body's endocrine system. They are excreted in small amounts (µg/d) in the bodily wastes both in their active form and as less active - but more soluble - conjugated metabolites (sulphates and glucuronides) (D'Ascenzo et al., 2003). Synthetic oestrogens like 17 α-ethinyloestradiol (EE2), a principal ingredient in birth control pills (Ternes et al., 1999), and mestranol (ME2) are used in medicine. Substances excreted in bodily wastes eventually appear in the wastewater stream, entering treatment plants at ng/ℓ concentrations (Johnson et al., 2000). Other environmental oestrogens include phenolic compounds which may be present in wastewater treatment plants and effluents in concentrations of high µg/ℓ (Giger et al., 1987; Ahel et al., 1994), up to 2 to 3 orders of magnitude higher than steroid oestrogens. Elimination of oestrogens within the treatment plant is therefore an important objective.

Yeast-based oestrogen receptor-transcription activation (ERTA) assays like the yeast oestrogen screen (YES) and the recombinant cell bioassay (RCBA) have shown a potential for use in screening for oestrogens, detecting and measuring oestrogenic activity and oestrogenic potency in samples of various origin (Routledge and Sumpter, 1996; Coldham et al., 1997; Aerni et al., 2004; Rutishauser et al., 2004). Oestrogen binds to the oestrogen receptor (ER) in yeast cells exposed to it. The estrogen-ER complex binds to oestrogen response elements (EREs) on the reporter plasmid initiating the transcription of *lacZ* gene mRNA, leading to production of β-galactosidase. This enzyme allows the cells to use lactose and lactose-analogs as a substrate. A chromogenic substrate like *ortho*-nitrophenyl-β-D-galactopyranoside (ONPG) is hydrolysed to galactose and yellow ortho-nitrophenol (ONP). The colour intensity is proportional to the concentration of ONPG hydrolysed and is related to the enzyme activity expressed and, in turn, to the exposure of yeast to oestrogen-like activity. ERTAs are sensitive to E2 and its metabolites, and oestrogen mimics (Coldham et al., 1997) and therefore measure total oestrogenic activity in a sample (Tanaka et al., 2001; Nakada et al., 2004; Servos et al., 2005). An ERTA response is then the total effect of a sample containing both E2 and its metabolites which bind to the oestrogen receptor with less affinity. Studies of mixtures of oestrogenic compounds are growing (Kortenkamp and Altenburger, 1999; Payne et al., 2000; Rajapakse et al., 2002; Silva et al., 2002) and while the complexity of interactions between E2, its metabolites, and the oestrogen receptor in a mixture is unknown at this stage, their overall effect can be estimated by the ERTA response. The ability to measure this important parameter readily can assist in monitoring the effectiveness of total oestrogenic activity removal in AS treatment systems.

This paper presents the development of a yeast-based assay protocol capable of detecting and quantifying relative total oestrogenic activity in unextracted activated sludge (AS) super-

* To whom all correspondence should be addressed.
Current address: Lawrence Berkeley National Laboratory, Mail Stop 70A-3317, 1 Cyclotron Road, Berkeley, CA, 94720
☎ 1 510 486 7094; fax: 1 510 486 7152;
e-mail: EWozei@LBL.gov
Received 21 June 2005; accepted in revised form 11 April 2006.