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# Diagnostic investigation of steroid estrogen removal by activated sludge at varying solids retention time

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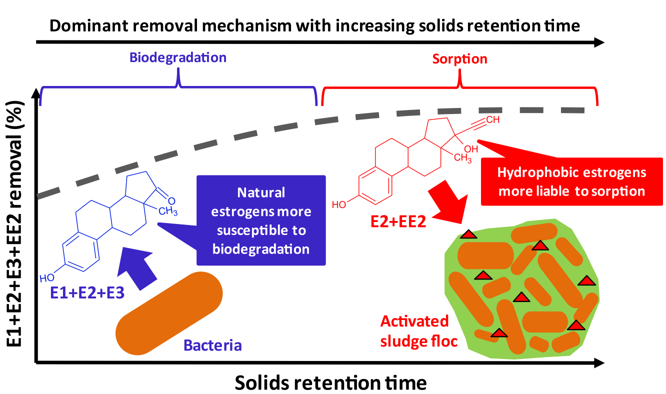
## HIGHLIGHTS

- Augmented estrogen removals (⩾90%) under nitrifying conditions.
- Removal not attributed to the activity of nitrifying bacteria.
- Increased estrogen biodegradation per viable bacterial cell with increasing SRT.
- Biodegradation by intracellular and/or extracellular enzymes contained within floc.
- Enhanced E2 and EE2 sorption to activated sludge at highest SRT.

## GRAPHICAL ABSTRACT

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## ABSTRACT

The impact of solids retention time (SRT) on estrone (E1), 17β-estradiol (E2), estriol (E3) and 17α-ethinylestradiol (EE2) removal in an activated sludge plant (ASP) was examined using a pilot plant to closely control operation. *Ex situ* analytical methods were simultaneously used to enable discrimination of the dominant mechanisms governing estrogen removal following transitions in SRT from short (3 d) to medium (10 d) and long (27 d) SRTs which broadly represent those encountered at full-scale. Total estrogen ($\sum_{EST}$, i.e., sum of E1, E2, E3 and EE2) removals which account for aqueous and particulate concentrations were 70 ± 8, 95 ± 1 and 93 ± 2% at 3, 10 and 27 d SRTs respectively. The improved removal observed following an SRT increase from 3 to 10 d was attributable to the augmented biodegradation of the natural estrogens E1 and E2. Interestingly, estrogen biodegradation per bacterial cell increased with SRT. These were 499, 1361 and 1750 ng $10^{12}$ viable cells$^{-1}$ d$^{-1}$. This indicated an improved efficiency of the same group or the development of a more responsive group of bacteria. In this study no improvement in absolute $\sum_{EST}$ removal was observed in the ASP when SRT increased from 10 to 27 d. However, batch studies identified an augmented biomass sorption capacity for the more hydrophobic estrogens E2 and EE2 at 27 d, equivalent to an order of magnitude. The lack of influence on estrogen removal during pilot plant operation can be ascribed to their distribution within activated sludge being under equilibrium. Consequently, lower wastage of excess sludge inherent of long SRT operation counteracts any improvement in sorption.



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## 1. Introduction

The fate and behaviour of steroid estrogens in wastewater treatment works (WwTWs) is of great importance due to their detrimental environmental impact (Lai et al., 2002a,b) and possible adverse implications for water re-use (Martin et al., 2008). This is a consequence of their high estrogenic potency (Kidd et al., 2007), and ubiquity in municipal wastewaters (Joss et al., 2004; Clara et al., 2005; Koh et al., 2009; McAdam et al., 2010). Natural estrogens; estrone (E1), 17β-estradiol (E2) and estriol (E3) constitute the majority (typically ⩾ 99%) of free steroid estrogens observed in wastewaters (Koh et al., 2009; McAdam et al., 2010). The synthetic estrogen 17α-ethinylestradiol (EE2) is present in much lower concentration (Koh et al., 2009; McAdam et al., 2010) but is arguably of greater concern due to its higher potency and the difficulty it poses to removal during conventional wastewater treatment. Such is the concern both E2 and EE2 were proposed to be included as priority hazardous chemicals under the Water Framework Directive (of the European Union) with suggested Environmental Quality Standards (EQS) of 0.4 ng $L^{-1}$ and 0.035 ng $L^{-1}$, respectively (European Commission, 2012). However, as currently configured secondary WwTWs fail to consistently meet these proposed guidelines (Koh et al., 2009; McAdam et al., 2010). Activated sludge is a widely implemented secondary biological process with an established ability to remove numerous anthropogenic (Stoveland et al., 1979; Petrie et al., 2013a) and complex natural chemicals including estrogens to varying extents (Kreuzinger et al., 2004; Clara et al., 2005; Koh et al., 2009; McAdam et al., 2010). To ensure effluent quality exceeds proposed EQS's by activated sludge, optimisation of process operation is necessary. Achieving this relies on understanding the mechanisms which drive removal at different operations. An extended solids retention time (SRT) is generally considered necessary to achieve enhanced removal of estrogens (Kreuzinger et al., 2004; Clara et al., 2005; Koh et al., 2009; McAdam et al., 2010). It has been considered that an increase in SRT enables the enrichment of a more diverse biocenosis which augments steroid estrogen biodegradation (Kreuzinger et al., 2004). It has also been suggested that the *k*-strategist concept may be applicable (Graham and Curtis, 2003; Koh et al., 2009), hypothesising that micro-organisms characterised by low half saturation co-efficients biodegrade estrogens at extended SRTs. The activity of nitrifying micro-organisms has long been associated with improved estrogen removal (Vader et al., 2000). However, the role of nitrifiers in environmentally representative conditions has been questioned (Gaulke et al., 2008), and it has been proposed that heterotrophic bacteria scavenging a broad spectrum of organics perform estrogen biodegradation in conditions conducive to nitrification (Bagnall et al., 2012).

Despite extensive research, the site of steroid estrogen biodegradation within the biomass matrix remains unknown. It is hypothesised that biological removal can occur as a free extracellular process within the bulk medium or, on the surface and/or within the activated sludge floc itself (Joss et al., 2004; Bagnall et al., 2012). Nitrifiers are known to produce ammonia mono-oxygenase enzymes capable of co-oxidising a variety of organic chemicals (Vader et al., 2000). Khunjar and Love (2011) suggested a requirement for sorption to occur prior to biological breakdown being initiated. Pharmaceuticals which failed to sorb to extracellular polymeric substances (EPS) were not biodegraded (Khunjar and Love, 2011). Thus sorption may be a prerequisite to the biodegradation process. Chemical hydrophobicity (log $K_{ow}$) is considered a reasonable predictor of sorption equilibrium (Gomes et al., 2011). McAdam et al. (2010) highlighted the possible importance of changing floc physiology with SRT to estrogen sorption. Activated sludge biomasses ranging in age from 4 to 20 d have been observed to differ in their surface properties such as charge and, EPS and protein content (Liao et al., 2001). Both EPS and proteins concentration are thought to influence EE2 sorption. For example, when EPS was removed EE2 sorption coefficients were reduced by ~50% (Khunjar and Love, 2011). Furthermore, EE2 was shown to sorb preferentially to EPS protein over carbohydrate. Activated sludge floc size is also likely to impact estrogen uptake as their size typically varies from 100 to 500 μm (Joss et al., 2004). To our knowledge no previous study has examined the impact of closely controlled SRT under the range examined in this paper to estrogen removal in municipal wastewater. Research has traditionally compared full-scale activated sludge plants (ASPs) where receiving sewage composition differs between sites and process control is poor due to fluctuations in receiving sewage flow (Kreuzinger et al., 2004; Clara et al., 2005; Koh et al., 2009; McAdam et al., 2010). The traditional approach of measuring SRT based on suspended solids (SS) measurements at full-scale ASPs has shown large errors. For example, a calculated SRT of 36 d for a full-scale works had a standard deviation of 22 d (Puig et al., 2008). This is attributed to variations in flow and settled solids concentrations of waste activated sludge (WAS) and return activated sludge (RAS) streams (Meijer et al., 2001). The implication is a high degree of uncertainty with respect to SRT, making the interpretation of its impact to estrogen removal difficult. On the other hand, previous research which has achieved process control by the operation of a pilot-scale ASP uses synthetic sewage which cannot replicate environmental characteristics of a real system (Clara et al., 2005; Bagnall et al., 2012). This study specifically examined the impact of SRT (3, 10 and 27 d) on steroid estrogen (E1, E2, E3 and EE2) removal from municipal wastewater using a highly controlled pilot-scale ASP. Supportive analysis including batch experiments and biomass characterisations were used to investigate sorption and biodegradation behaviour of steroid estrogens whilst at constant temperature and normalised biomass solids concentrations at each SRT studied.

## 2. Materials and methods

### 2.1. Pilot-scale activated sludge plant

A pilot-scale ASP was sited at a WwTWs in the east of England (3000 population equivalent – PE) and consisted of a primary sedimentation tank, a 0.36 $m^3$ aerated basin and a final clarifier (Fig. 1). The system was seeded using biomass from a full-scale nitrifying ASP (280 000 PE) and continuously supplied with municipal crude wastewater from the 3000 PE (Petrie et al., 2013b,c) site which contained indigenous concentrations of estrogens. The influent flow rate (1.08 $m^3$ $d^{-1}$) was controlled to achieve a constant hydraulic retention time (HRT) of 8 h. RAS was 0.55 of the influent flow (0.59 $m^3$ $d^{-1}$). SRTs of 3, 10 and 27 d were selected for monitoring and controlled by daily disposal of excess WAS following correction for loss of effluent solids. The system was operated for at least three SRTs prior to monitoring to ensure steady state conditions were established. Steroid estrogens and sanitary determinands were sampled once daily from influent settled sewage, final effluent and RAS (in duplicate) over seven consecutive d at each condition. Grab samples was employed in this study due to the possible biodegradation of trace organic contaminants during sampling (Baker and Kasprzyk-Hordern, 2013). Samples were collected in 2.5 L borosilicate glass vessels with Teflon lined caps and processed immediately. During sampling no significant rainfall was experienced.