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Removal of natural hormones in dairy farm wastewater using reactive and sorptive materials

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HIGHLIGHTS

- Reactive and sorptive materials removed natural hormones from dairy wastewater.
- Connelly ZVI and OrganoLoc PM-100 OC gave a maximum and fastest removal of oestrogens.
- OrganoLoc PM-100 OC and AquaSorb 101 GAC were most suitable in removing androgens.
- OrganoLoc PM-100 OC presented a maximum removal of oestrogens and androgens.
- Reactive and sorptive materials may enhance hormone removal in treated wastewater.

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ABSTRACT

The objective of this study was to examine the oestrogen and androgen hormone removal efficiency of reactive (Connelly zero-valent iron (ZVI), Gotthart Maier ZVI) and sorptive (AquaSorb 101 granular activated carbon (GAC) and OrganoLoc PM-100 organoclay (OC)) materials from HPLC grade water and constructed wetland system (CWS) treated dairy farm wastewater. Batch test studies were performed and hormone concentration analysis carried out using highly sensitive reporter gene assays (RGAs). The results showed that hormonal interaction with these materials is selective for individual classes of hormones. Connelly ZVI and AquaSorb 101 GAC were more efficient in removing testosterone (Te) than 17β-estradiol (E2) and showed faster removal rates of oestrogen and androgen than the other materials. Gotthart Maier ZVI was more efficient in removing E2 than Te. OrganoLoc PM-100 OC achieved the lowest final concentration of E2 equivalent (EEQ) and provided maximum removal of both oestrogens and androgens.

1. Introduction

Endocrine disrupting compounds (EDCs) are of concern because they may alter the normal function of the endocrine system in humans and wildlife (Colborn et al., 1993; Vos et al., 2000; Orlando et al., 2004; Robertson et al., 2009) and when released into the environment lead to effects such as the feminisation of male fish (Sumpter, 1995).

Natural oestrogens are of particularly high concern as low parts per trillion concentrations (10–100 ng/L) have been shown to have an effect on the levels of the oestrogenic exposure biomarker, vitellogenin, in fish (Routledge et al., 1998). Consequently lowest observable effect concentrations (LOECs) have been proposed for the natural oestrogen 17β-estradiol (E2) at 10 ng L^{-1} and a predicted no effect concentration (PNEC) of 1 ng/L (Young et al., 2002). *In vivo* testing has demonstrated that the sexual differentiation of fish can be influenced by both exogenous oestrogens (Tabata et al., 2001; Jobling et al., 2004) and androgens (Katiadaki et al., 2002; Margiotta-Casaluci and Sumpter, 2011). However the number of studies on environmental androgens to date is fewer than oestrogens.

More recently agriculture has been highlighted as an important source of natural steroid oestrogens. Urinary and faecal excretion rates in a range of farm animals have been calculated in studies in the UK (Johnson et al., 2006) and EU and USA (Lange et al., 2002). These studies have highlighted dairy cows as the largest contributor of excreted oestrogens in comparison to pig, sheep and chickens

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(Johnson et al., 2006; Lange et al., 2002) and humans (Johnson et al., 2006). Many agricultural wastes are applied as a fertiliser and spread on land. Although there is evidence for localised contamination of surface and ground waters associated with cattle operations and manure applications to land, calculations based on the high sorption affinity of soils for oestrogens and their short half-life suggest that soils should retain >99% of oestrogenic load to soils (Johnson et al., 2006).

However, another class of agricultural waste termed "wastewater" can be defined as dilute washings from dairy and milking parlours and run-off from lightly contaminated yard areas to which animals have regular access (DEFRA, 2009). Wastewater offers minimal agronomic return as a fertiliser due to its dilute nature and land spreading is economically unfavourable. A number of studies (Gadd et al., 2010; Kolodziej et al., 2004; Arnon et al., 2008; Sarmah et al., 2006; Zheng et al., 2008) have reported that E2 and/or Testosterone (Te) are frequently detected in dairy farm wastewater from Concentrated Animal Feeding Operations. This may also be the case for wastewaters from smaller dairy farms.

Overall, the removal of hormones from dairy farm wastewater before discharge is a new challenge for the protection of water resources. Constructed wetland systems (CWSs) have been used as a treatment system for agricultural wastewater (Schaafsma et al., 2000; Harrington et al., 2007; Healy et al., 2007). There are limited studies on the efficiency of CWSs in their removal of hormones from wastewater. Song et al. (2009) report the highest removal efficiency in one type of CWS as 67.8 ± 28.0%, 84.0 ± 15.4% for oestrone (E1) and E2, respectively. A more recent study on dairy farm wastewater treatment *via* a surface flow CWS reported up to 98.6% and 96.2% removal efficiency of oestrogens and androgens respectively, with final outflow concentrations ranging between 0.3 ng/L up to a maximum of 18.8 ng/L E2 equivalent (EEQ) and 8.7 ng/L up to a maximum of 110 ng/L Te equivalent (TEQ) (Cai et al., 2012). Therefore, residual hormone levels may be present and advanced treatment processes after CWS treatment may be required to meet the LOEC or PNEC standard.

Sorptive and reactive materials such as zero-valent iron (ZVI) have previously been used for ground water remediation in permeable reactive barriers (Phillips et al., 2010; Noubactep and Caré, 2010). There is a great deal of interest in the degradation of aquatic contaminants using ZVI due to its applicability under different geochemical conditions, ease of use and low cost maintenance (Tyrovola et al., 2007). Various forms of ZVI include Connelly Iron, Gotthart and Maier Iron, Master Builders ZVI and Peerless ZVI. Although numerous studies have been carried out on the treatment of organic and inorganic contaminants in aqueous solution by ZVI (Tyrovola et al., 2007; Shin et al., 2008; Mu et al., 2004), no research has been conducted on the efficiency of its removal of naturally occurring hormones.

Granular activated carbon (GAC) is a sorptive material well known for the removal of EDCs and pharmaceuticals (Kim et al., 2007; Zhang and Zhou, 2005). Sorption experiments have demonstrated that GAC has a very high sorption capacity with a maximum sorption constant of 9290 mL/g for oestrone and 12,200 mL/g for E2 (Zhang and Zhou, 2005). However, studies concerned with the removal of androgens using GAC, such as that of Stalter et al. (2011), are few and only beginning to emerge.

Clay is of interest as a solid catalytic matrix constituted of various mineral ions having the potential to act as catalytically active reagents (Varma, 2002). Previous reports of effective sorption by organoclay (OC) of lipophilic substances (Gianotti et al., 2008) and the oestrogenic endocrine disruptor zearalenone (Lemke et al., 1998; Ryu et al., 2003) led to the hypothesis that this technology could be useful in binding EDCs such as oestrogen and androgen. These sorption studies have shown that OC can effectively bind to zearalenone by attracting zearalenone into the clay's interlayer where at a neutral pH, zearalenone binds to the hydroxyl group of the clay *via* ion–dipole interaction and electrostatic attraction to the excess exchanged surfactant cations (Lemke et al., 1998; Ryu et al., 2003).

The detection and monitoring of EDC activity may be achieved at extremely low concentrations (ppt) with reporter gene assays (RGAs) utilising stable reporter cell lines containing relevant hormone receptors. Consequently RGAs have previously been applied in detecting hormonal activity in environmental samples such as sediment (Houtman et al., 2007), wastewater and surface water (Blankvoort et al., 2005) and CWS treated dairy farm wastewater (Cai et al., 2012).

The overall aim of this project was to determine, through monitoring with RGAs, the oestrogen and androgen hormone removal efficiency of a variety of sorptive and reactive materials from dairy farm wastewater.

2. Material and methods

2.1. Chemicals and reagents

All chemicals and reagents were supplied by BDH (Poole, Dorset, UK) unless otherwise stated. Water chromasolv® for HPLC was bought from Sigma-Aldrich, UK. Reference standards E2 and Te were obtained from Sigma-Aldrich, UK. Standards were prepared at a concentration of 1 mg/mL in 100% methanol and were stored at −20 °C to minimise evaporation and degradation. A Promega Luciferase Assay system (Cat. 1501) was obtained from MSC Ltd., Ireland. GIBCO® cell culture reagents including Dulbecco's Modified Eagle Medium (DMEM) (with/without phenol red), penicillin, streptomycin, fetal bovine serum and hormone-depleted serum were obtained from Invitrogen Ltd., UK. Thiazolyl blue tetrazolium bromide (MTT) was obtained from Sigma-Aldrich (Poole, Dorset, UK). Connelly and Gotthart Maier zero valent iron (ZVI) was obtained from Connelly-GPM, Inc., Chicago, IL and Gotthart Maier Metallpulver GmbH (Rheinfelden, Germany), respectively. OrganoLoc PM-100 Organoclay (OC) and AquaSorb 101 granulated activated carbon (GAC) were obtained from Amcol Speciality Minerals, Winsford, Cheshire, England and Jacobi Carbons, Ltd., Leigh Lancs, respectively. Mesh size for the materials was as follows: 0.2–3 mm (Connelly ZVI), 0.2–8 mm (Gotthart Maier ZVI), 0.2–2 mm (OrganoLoc PM-100 OC), 0.425–1.7 mm (AquaSorb 101 GAC). The BET method (Brunauer et al., 1938) was used to measure specific surface area (SSA) on these materials at the Analytical Services and Environmental Projects (ASEP) in the School of Chemistry and Chemical Engineering at Queen's University of Belfast, Belfast, Northern Ireland. BET values of the materials are 1.3 m^2/g (Connelly ZVI), 0.6 m^2/g (Gotthart Maier ZVI), 900 m^2/g (AquaSorb 101 GAC), and 7.24 m^2/g (OrganoLoc PM-100 OC).

2.2. Sample collection

Dairy farm wastewater (1 L) was collected from the first treatment pond of a surface flow CWS located in north east Ireland (Lat. 54°42′ 02.06″N and Long 6°12′39.62 W) which is connected to a dairy farm research facility at the College of Agriculture, Food and Rural Enterprise (CAFRE) (Greenmount campus), Northern Ireland (Forbes et al., 2011; O'Neill et al., 2011). Batch-tests were carried-out on these samples immediately after collection to minimise biotransformation by microbes.

2.3. Batch test analysis

All experiments were performed in triplicate for each experimental point and repeated in 3 independent experiments.

Prior to batch test analysis, RGA results confirmed that HPLC water was negative for hormone activity and dairy farm wastewater was found to contain 51.5 ng/L EEQ and 123.96 ng/L TEQ. Calculations were adjusted for the removal of sample by normalizing each point as (original concentration of sample * volume of sample after removal − total volume removed from original volume) / volume of sample after removal. Unspiked HPLC grade water/wastewater was used as a negative control and spiked HPLC grade water/wastewater without any sorptive and reactive materials was included as a positive control.