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Assessment of the removal of estrogenicity in biological nutrient removal wastewater treatment processes

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HIGHLIGHTS

- Comparable estrogenicity removal was observed from two BNR processes.
- Pseudo first order model described the transformation of E2 and E1 in BNR process.
- Biotransformation of E1 in BNR activated sludge controls the degradation of E2.

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ABSTRACT

The removal of estrogenicity in a University of Cape Town-biological nutrient removal (UCT-BNR) wastewater treatment process was investigated using pilot and bench scale systems, batch experiments and mathematical modeling. In the pilot BNR process, 96 ± 5% of the estrogenicity exerted by the influent wastewater was removed by the treatment process. The degradation efficiencies in the anaerobic, anoxic and aerobic zones of the pilot BNR bioreactor were 11 ± 9%, 18 ± 2% and 93 ± 10%, respectively. In order to further understand the performance of the BNR process in the removal of estrogenicity from wastewater, a bench scale BNR process was operated with synthetic wastewater dosed with E1 and E2. The removal of estrogenicity in the bench scale system (95 ± 5%) was comparable to the pilot BNR process and the degradation efficiencies were estimated to be 8 ± 0.8%, 38 ± 4% and 85 ± 22% in the anaerobic, anoxic and aerobic zones, respectively. A biotransformation model developed to predict the fate of E1 and E2 in batch tests using the sludge from the BNR process was calibrated using the data from the experiments. The biotransformation rate constants for the transformation of E2 to E1 were estimated as 71 ± 1.5, 31 ± 3.3 and 1 ± 0.9 L g COD^{-1} d^{-1} for the aerobic, anoxic and anaerobic batch tests, respectively, while the corresponding biotransformation rate constants for the transformation of E1 were estimated to be 7.3 ± 1.0, 3 ± 2.0, and 0.85 ± 0.6 L·g COD^{-1} d^{-1}. A steady state mass balance model formulated to describe the interactions between E2 and E1 in BNR activated sludge reasonably described the fate of E1 and E2 in the BNR process.

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1. Introduction

Effluents from wastewater treatment plants (WWTPs) have been shown to contain a mix of endocrine disrupting compounds (EDCs) that could induce physiological effects either individually or synergistically on aquatic organisms (Desbrow et al., 1998; Sumpter, 1998; Nakada et al., 2004; Vajda et al., 2011; Wojnarowicz et al., 2013; Parker et al., 2014). The USEPA has defined EDCs as exogenous agents that interfere with the synthesis, secretion, transport, binding action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behavior. Some of the effects of EDCs on aquatic organisms include reduced reproductive capacity and vitellogenin production (precursor of egg yolk protein) in male fish, decreased female fish fertility and survival of juveniles, reduced fish egg fertilization and thyroid hormone disruption in tadpoles (Purdom et al., 1994; Jobling and Sumpter, 1993, 1998; Andersen et al., 2003; Vajda et al., 2011; Wojnarowicz et al., 2013; Parker et al., 2014).

Synthetic and natural EDCs enter sewer systems through human and animal excretions (Combalbert and Hernandez-Raquet, 2010). Stringent policies could be formulated by regulatory agencies to attenuate the risks associated with EDCs in the environment. However, the anthropogenic release of these substances into the environment is difficult to control because some of these compounds are naturally produced in the human or animal body. For example, the natural estrogens, E1 and E2 excreted by pregnant women could be as high as 600–940 and 170–330 μg/day/person, respectively (Johnson et al., 2000). Hence, the removal of EDCs in wastewater treatment processes will be required for attenuating their release into the aquatic environment.

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Biological nutrient removal (BNR) wastewater treatment processes are advanced configurations that provide carbon, nitrogen and phosphorus removal. The removal and biodegradation of macropollutants in BNR processes is well documented but the fate of EDCs that are prevalent in wastewater in these processes is less well understood. Previous studies that have investigated the removal of EDCs in BNR wastewater treatment processes have reported greater than 90% removal efficiencies of the compounds (Koh et al., 2009; Li et al., 2011). However, it is still unclear whether the high removal of EDCs in BNR treatment processes can be translated into a high reduction in estrogenic responses through the systems. A recent study that compared the removal of estrogenicity in conventional activated sludge (CAS), nitrifying activated sludge (NAS) and biological nutrient removal (BNR) processes showed greater than 80% estrogenicity removal in all processes with the highest removal in the BNR treatment process (Ogunlaja et al., 2013). However, the impact of the different stages of treatment on estrogenicity reduction was not examined in detail.

The quantification of the estrogenic potency of EDCs in WWTPs is not trivial because EDCs exist as a cocktail in WWTPs influents and effluents. The potential for synergistic action of the mixture of EDCs in wastewater has challenged previous attempts to relate calculated EDC concentrations with measured estrogenicity in WWTPs (Petrovic et al., 2004). Therefore, in order to give a holistic assessment of the estrogenicity of a WWTP effluent, previous studies have employed *in vitro* bioassays to augment chemical measurements (Servos et al., 2005; Wu et al., 2011; Parker et al., 2014).

In general, there have been few studies that have employed bioassays to investigate estrogen biodegradation in BNR activated sludge. Previous studies that have investigated the biodegradation of EDCs in activated sludge systems have monitored the disappearance of the compounds using chemical techniques without an understanding of the estrogenicity associated with the disappearance of the compounds (Joss et al., 2004; Dytczak et al., 2008). However, it has been demonstrated in other process configurations that the disappearance of estrogenic compounds does not necessarily eliminate estrogenicity. For example, the transformation of 17β-estradiol (E2) in activated sludge processes was reported to involve E2 oxidation to estrone (E1), another estrogenic compound (Ternes et al., 1999; Shi et al., 2004; Dytczak et al., 2008). There is the potential for differing conversions between estrogenic compounds in different redox conditions (Joss et al., 2004; Czajka and Londry, 2006; Dytczak et al., 2008). Hence, bioassays are important tools for characterizing the impact of redox conditions on the fate of estrogenicity in BNR processes.

This study employed the YES assay technique to investigate the removal and biotransformation of EDCs in BNR activated sludge. In the current study, E1 and E2 were evaluated as target EDCs because several studies have shown that E1 and E2 constitute a substantial fraction of the dominant estrogens found in the effluents of WWTPs (Nakada et al., 2004; Aerni et al., 2004; Fernandez et al., 2007; Muller et al., 2008). Specifically, this study employed the recombinant yeast screen to 1) investigate the removal of estrogenicity in BNR processes operated with both authentic and synthetic wastewaters 2) estimate the biotransformation rate constants for E1 and E2 in aerobic, anoxic and anaerobic batch reactors, and 3) investigate the transformation kinetics between E2 and E1 under anaerobic, anoxic and aerobic conditions.

2. Materials and method

2.1. Pilot scale BNR wastewater treatment process

A detailed description of the UCT-BNR pilot plant was described elsewhere (Ogunlaja, 2015). The operating and design conditions of the pilot plant are summarized in Table 1 while Fig. 1 presents the flow schematics of the pilot BNR process. The pilot UCT-BNR process was operated on authentic municipal wastewater that was augmented with stock solutions of sodium bicarbonate to provide alkalinity, dipotassium phosphate as phosphorus source and sodium acetate to enhance the proliferation of PAOs. The resultant influent concentrations of COD, alkalinity and total phosphorus were 367 ± 48 mg/L, 268 ± 21 mg/L and 11 ± 7 mg/L, respectively. The pH range of the authentic wastewater entering the bioreactor was 7.5–8.2.

Table 1
Pilot BNR operating and design conditions.

Unit	Size/description	Unit
Flow rate	1.3	m^3/d
Primary clarifier	Area = 0.46	m^2
	Depth = 1.56	m
Bioreactor	Volume = 0.36	m^3
	Depth = 1.28	m
	DO(aerobic) = 4–5	g/m^3
	DO(anoxic) = 1–2.5	g/m^3
	DO(anaerobic) = 0–0.2	g/m^3
	NO_3 (aerobic) = 6 ± 2	gN/m^3
	NO_3(anaerobic) = 0	gN/m^3
Final clarifier	Area = 0.204	m^2
	Depth = 1.4	m
Recycle rate	Aerobic = 2.6	m^3/d
	Anoxic = 1.3	m^3/d
SRT	20	d
Aerobic SRT	10	d
RAS flow rate	0.9	m^3/d
Waste rate	0.018	m^3/d
Nominal HRT	7	h
Temperature	18 ± 2	°C

RAS — return activated sludge, SRT — solid residence time, HRT — hydraulic retension time.

2.2. Bench scale BNR wastewater treatment process

The bench scale UCT-BNR process consisted of 3–10 L coupled reactors made of acrylic plastic and a 25 L final clarifier for solid–liquid separation (supporting information (SI) Figure S1). The aerobic reactor was mixed and aerated with fine bubble aerators while the anaerobic and anoxic reactors were mechanically mixed. The influent flow to the system was maintained at 0.086 ± 0.01 m^3/day and the HRT was 5 h. The return activated sludge was operated at 75% of the influent flow rate and the internal recycle ratios were 200% and 100% of the influent flow rate for the aerobic and anoxic recycles respectively. Approximately 0.9 ± 0.1 L/d of sludge was wasted from the aerobic zone of the bioreactor to maintain a total SRT of 20 days. The DO in the aerobic zone was maintained at 5–7 mg/L and the temperature of the system was maintained at 20 ± 2 °C. The operational parameters of the bench scale BNR process were consistent with typical operational parameters of commonly used BNR processes (WEF, 2005).

The bioreactors were inoculated with mixed liquor collected from the return activated sludge stream of a full scale BNR WWTP in southern Ontario. The composition of the synthetic wastewater that was fed to the system is presented in SI Table S1. The system was initially maintained without addition of E1 and E2 into the influent stream for one SRT to allow acclimatization of the biomass to the synthetic feed. Subsequently, a volume of E2 and E1 dissolved in water was dosed into the synthetic wastewater with the objective of achieving a target concentration of 100 ng/L for E1 and E2. These dosed concentrations were higher than those typically observed in Canadian wastewater (Servos et al., 2005). However, this concentration enabled measurement of the estrogenicity in the samples, considering the sample matrix. After two months of operation, steady state was achieved and the aerobic bioreactor mixed liquor with an MLVSS concentration of 3817 ± 150 g MLVSS/m^3 was employed for the batch tests.

2.3. Monitoring and sampling-pilot and bench scale BNR system

Twenty four hour composite samples of the pilot plant influent and effluent were collected in pre washed stainless steel containers, three times a week for two weeks using a refrigerated autosampler for

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