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Biodegradation of natural and synthetic estrogens in moving bed bioreactor

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ABSTRACT

Estrogen hormones as a group of endocrine disruptive compounds (EDC) can interfere with endocrine system in humans and animals. The goal of this study was to investigate the elimination rate of Estrone (E1), 17β-estradiol (E2) and 17α-ethinyl estradiol (EE2) in Moving Bed Bioreactor (MBBR). These analytes extracted by Dispersive Liquid–Liquid Microextraction (DLLME) technique, followed by derivatization, and detected by GC/MS. Estrogen removal efficiency in MBBR improved at high solid retention times (SRTs), which notion is owing to development of nitrification. Estrogen specific removal rate was between 0.22–1.45 $\mu g \cdot (gVSS)^{-1} \cdot d^{-1}$ for natural and synthetic hormones. The adsorption rate was 0.9%–3.2%, 0–1.3%, and 0.7%–5.7% for E1, E2, and EE2, respectively. In addition, the biodegradation rates were more than 95% for these compounds. These results illustrated that in MBBR, the biodegradation and the adsorption to biomass are considered as two significant routes for elimination of estrogenic compounds. As a whole, the deterioration rate of estrogens enhanced by MBBR compared to other biological wastewater treatment processes such as conventional activated sludge.

1. Introduction

Many regulatory agencies, such as WHO, USEPA and EU have listed EDC as priority pollutants [1–3]. Estrogenic hormones are a group of EDCs that can interfere with endocrine system in humans and animals [4,5]. Estrone (E1), 17β-estradiol (E2) Natural estrogens, and 17α-ethinyl estradiol (EE2) synthetic estrogen, have considered as substances, which have high potential for endocrine-disrupting effects. Many of wastewater treatment plants (WWTPs) do not have enough potential for elimination of micropollutants. Therefore, numerous studies have found the effluent of WWTPs as main sources for introduction of these compounds in aquatic environment [5–8]. Consequently, the occurrence of EDCs, menace health of humans and wildlife and inducement detrimental effects on aquatic life especially in receiving water [7,9,10]. Biodegradation, adsorption, air stripping and photolysis are four important mechanisms for EDC removal within wastewater treatment process [11–13]. Nevertheless, the biodegradation and the sorption are propound to be the most substantial contribution in decomposition of these compounds. Other studies which have been conducted in WWTPs, reported roughly 50%–95% of estrogenic compounds (E1, E2, and EE2) can be removed in conventional activated sludge plants [14–16]. Researchers announced the fate of EDCs in microbial degradation, affected by some operation parameters such as hydraulic retention time (HRT) and solid retention time (SRT), physicochemical characteristics of hormones and biomass particle [17,18]. SRT and HRT are two most crucial operational parameters in biological wastewater treatment processes, particularly in a nitrification/denitrification process. At long SRT, slow growing bacteria such as nitrifying cultures, would have enough time to proliferation [19]. De Mes *et al.* reported that at high HRT and SRT, removal efficiency of estrogenic compounds can be enhanced [20]. In the work by Joss *et al.* the removal of E1, E2, and EE2 in WWTP was more than 90%, specifically in nitrification and denitrification processes which SRT was 12–15 days [21]. According to applied results of Koh *et al.*, which measured the mass balance in a nitrifying/denitrifying activated sludge plant at SRT > 13 days, the biodegradation rate of estrogens was 70% [22]. The biodegradability of natural and synthetic estrogens has been reported by Hashimoto and Murakami in an oxidation-ditch process. They represented that the degradation of E2 was achieved very fast at SRT > 15 days; while EE2 had lag phase of 2 h and the degradation was accomplished after 24 h [23].

Contrary to conventional and advanced wastewater treatment processes, the attached growth processes are effective and have many advantages over the suspended growth. The Moving Bed Bioreactor (MBBR), as an attached growth process is executable technology and has been recently gaining amicability. The inherent preponderance in the following aspect including, high biomass activity and redox conditions in biofilm improve the elimination of micropollutants, organic substances, nitrification and denitrification [24,25]. Ehsan Ahmadi reported that, more than 92% of phthalates removal was achieved in

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MBBR. Yunlong Luo *et al.* investigated the removal of micropollutants in a sponge-based MBBR. They determined that removal rate has been between 25.9% and 96.8% [25]. To our knowledge, previous experiments were not conducted for the identification of the effect of HRT and SRT for elimination rates of E1, E2, and EE2 in MBBR. This paper seeks to address the effect of these operational parameters (SRT and HRT) for removal of estrogens. The analytes were extracted by a preconcentration technique, Dispersive Liquid–Liquid Microextraction (DLLME), and detected by gas chromatography followed with mass spectrometry (GC–MS).

2. Materials and Methods

2.1. Reagents

All chemicals were of analytical reagent grade. The target steroidal compounds included Estrone (E1), 17β-estradiol (E2), 17α-ethinyl estradiol (EE2), pyridine and derivatization reagent N-O bis (trimethyl) trifluoroacetamide (BSTFA) containing 1% of trimethylchlorosilane (TMCS) were purchased from Sigma. MeOH of GC grade obtained from Merck. All glassworks are washed with nitric acid (6 $mol \cdot L^{-1}$) and then consecutive with deionized water before use. Separate stock solutions for E1 and E2 in 200 $mg \cdot L^{-1}$ and 100 $mg \cdot L^{-1}$ for EE2 supplied by dissolving an appropriate amount of each substance in methanol and were stored at 4 °C prior to use. The working solutions of specific standards were prepared through the serial dilution of stock solutions and pure deionized water.

2.2. MBBR system

The experiments conducted using the lab scale MBBR which schematic diagram presented in Fig. 1.

A cylindrical shaped reactor was used with an internal diameter of 150 mm and 500 mm in height, operating volume of 5 L and filled with 40% (determined from the batch experiments) of acclimatized carrier of floating media. Table 1 summarizes characteristics of the media.

Air diffusers and influent pipe were installed at the bottom of the reactor. The Coarse bubble diffusers had flowed for 4 min^{-1} and provided oxygen concentration between 4 and 5 $mg \cdot L^{-1}$ for bacterial activity and circulation of carriers. The sampling ports for sample collection were set in reactor. The reactor operated in an up-flow mode. Synthetic wastewater fed continuously from storage tank into bioreactor through pump (Etatron-Italy). Synthetic wastewater composition including glucose (600 $mg \cdot L^{-1}$) as carbon source and $(NH_4)_2SO_4$ (90 $mg \cdot L^{-1}$), KH_2PO_4 and K_2HPO_4 (9 $mg \cdot L^{-1}$) as nutrient was employed in all experiments. Also, the wastewater was enriched with the following components, as $mg \cdot L^{-1}$, including: 4.4 $CaCl_2 \cdot 2H_2O$, 12.2 $MgSO_4 \cdot 7H_2O$, 0.05 $MnCl_2 \cdot 4H_2O$, 0.132 $ZnSO_4 \cdot 7H_2O$, 18.2 $FeCl_3$, 0.01 $CuSO_4 \cdot 5H_2O$, 0.04 $CoCl_2 \cdot 6H_2O$, 0.15 $Na_2MoO_4 \cdot 2H_2O$, 0.054 KI and 0.045 H_3BO_3 [26]. To set up the system, reactor was acclimatized for microbial growth by synthetic wastewater without hormones and activated sludge within 120 days. Afterwards, synthetic wastewater was spiked with hormones at different organic loading rates and HRTs were continuously introduced to the bioreactor (Table 2). The elimination rate of natural and synthetic hormones was carried out over a period of 120 days. $NaHCO_3$ and NaOH were used to adjust the pH and alkalinity.

Table 1
Characteristics of the floating media

Material	Polypropylene
Shape	Cylindrical
Dimension	22 mm × 15 mm
Density	0.97 $g \cdot cm^{-3}$
Specific surface	400 $m^2 \cdot m^{-3}$

Table 2
Technical data and operation results for the moving bed biofilm reactor

Parameters	HRT/h			
	4	8	12	16
Organic loading rate/kg COD $\cdot m^{-3} \cdot d^{-1}$	3.3	1.65	1.1	0.75
Influent COD/$mg \cdot L^{-1}$	600	600	600	600
Effluents COD/$mg \cdot L^{-1}$	70	58	35	18
COD removal efficiency/%	88.3	90.3	94.16	97
Nitrification rate/%	51.93	64.2	70.4	84.8
Denitrification rate/%	40.4	44.2	47.06	50.4

2.3. Analytical methods

Analysis of soluble and chemical oxygen demand (sCOD) and rapid biodegradability chemical oxygen demand (rbCOD) in effluent, biosolids (as MLSS) and biomass as (MLVSS), NH_4-N, NO_2-N, NO_3-N, and PO_4-P was performed periodically according to standard methods [27]. The attached growth bio-solid concentration was determined by

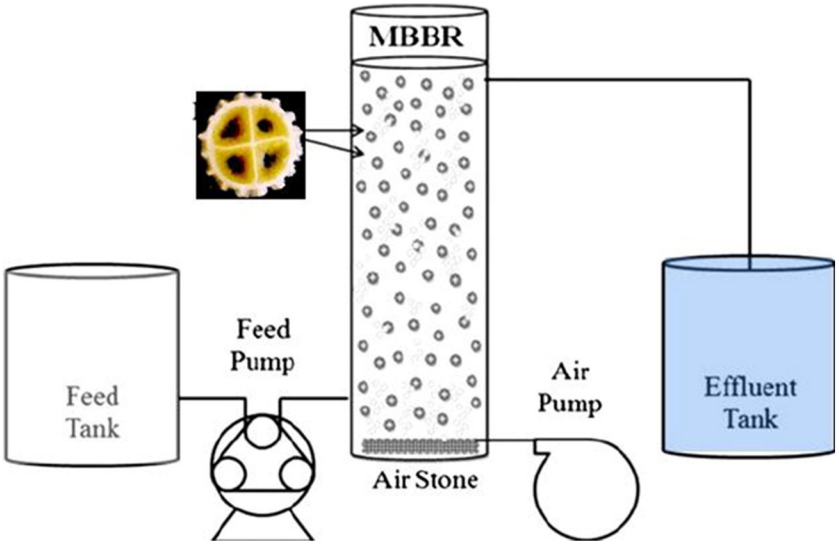

Fig. 1. Schematic diagram of the lab scale MBBR system.

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