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# Kinetic parameters for 17α-ethinylestradiol removal by nitrifying activated sludge developed in a membrane bioreactor

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ABSTRACT

The synthetic hormone 17α-ethinylestradiol (EE2) is primarily removed in wastewater treatment plants (WWTPs) by sorption, and nitrifying biomass has been shown to be responsible for EE2 biodegradation. Membrane bioreactor (MBR) technology was chosen to develop a community of autotrophic, nitrifying micro-organisms and determine kinetic parameters for EE2 biodegradation. Biological inhibition by azide was applied to differentiate sorption from biodegradation. Activated sludge (AS) was acclimated in the MBR to a substrate specific to autotrophic biomass and resulted in an increase in nitrifying activity. Acclimated AS was used to successfully biodegrade EE2 (11% increase in EE2 removal), and the overall removal of EE2 was determined to be 99% (sorption + biodegradation). AS used directly from a WWTP without acclimation removed EE2 only through sorption (88% removal of EE2). Therefore, higher nitrifying activity developed by acclimating AS allowed almost complete removal of EE2.



## 1. Introduction

The synthetic hormone 17α-ethinylestradiol (EE2) is an endocrine disrupter that has caused feminization in several species among fish (Mills and Chichester, 2005), molluscs (Ketata et al., 2008), amphibians (Gyllenhammar et al., 2009), birds (Brunström et al., in press) and mammals (Latendresse et al., in press), at concentrations as low as 0.1 $ng_{EE2}\,L^{-1}$ (Purdom et al., 1994). This xenobiotic was classified as at least R51/53 which means the compound is "toxic to aquatic organisms and may cause long-term effects in the aquatic environment" (Carlsson et al., 2006). EE2 is thus of great concern as a water contaminant. *Pimephales promelas* was exposed to EE2 (5–6 $ng_{EE2}\,L^{-1}$) during a whole-lake experiment and the exposure induced feminization in the males, followed by a near extinction of this fish species (Kidd et al., 2007).

EE2 occurrence in the environment is due to insufficient removal of the compound by municipal wastewater treatment plants (WWTPs) (Clouzot et al., 2008). Contamination of receiving waters has been measured up to 100 km downstream from WWTPs, with concentrations sufficient to induce endocrine disruption (>1.5 $ng_{EE2}\,L^{-1}$) (Barel-Cohen et al., 2006). Sorption is the process that is primarily responsible for EE2 removal in WWTPs; the typical decrease in aqueous EE2 concentration due to sorption is between 60% and 80% (Andersen et al., 2005; Johnson and Sumpter, 2001). However, sorption is a pollutant transfer process, and a significant amount of the EE2 passes through the wastewater treatment processes; only biodegradation allows complete removal of the contaminant. Nitrifying biomass has been shown to biodegrade EE2 by co-metabolism of the enzyme ammonium monooxygenase (AMO) (Shi et al., 2004; Vader et al., 2000). A linear trend between nitrifying activity and EE2 removal has been established (Yi and Harper, 2007). However, for initial ammonium concentrations below 50 $mg^{+}_{NH_4}\,L^{-1}$, sorption was shown to be the predominant removal mechanism (up to 60%) because of low co-metabolic activity. For higher ammonium concentrations, biodegradation became more important (up to 50%) (Yi et al., 2006). Therefore, for relevant ammonium concentrations in WWTPs (in the low $mg\,L^{-1}$ range), sorption plays a more significant role than biodegradation (Johnson and Sumpter, 2001).

Nitrifying micro-organisms are autotrophic and grow more slowly than heterotrophic ones. Therefore, heterotrophic micro-organisms typically outnumber nitrifying micro-organisms. The development of nitrifying micro-organisms during wastewater treatment can be improved with high sludge retention times (SRT). In a conventional activated sludge (AS) system, low settling abilities of sludge generally result in low SRT (15–20 days). However, with a membrane bioreactor (MBR), complete biomass retention allows control of a higher SRT. Based on a biological model, EE2 removal was predicted to be more efficient with acclimated, nitrifying AS from a MBR (SRT = 30 days) than with AS from a

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conventional WWTP (SRT = 11 days) (Joss et al., 2004). Autotrophic nitrifying biomass requires specific conditions for growth, and the organisms have a high sensitivity to external parameters such as pH, dissolved oxygen concentration, and temperature. Typical required conditions include a pH of 7.5, a dissolved oxygen concentration above 4 $mg_{O_2}$ $L^{-1}$ and a temperature range of 30–36 °C. The MBR system configuration can also influence performance and degradation; in particular, immersed configurations are characterized by lower shear stress than external configurations. Thus, an immersed MBR was determined to be more appropriate for nitrifying micro-organism growth. However, operating conditions of an immersed MBR are known to induce higher membrane fouling. Recent work has resulted in the development of a new external-immersed MBR configuration (the membrane is immersed in an external carter), which allows improved fouling control in the membrane system by air sparging (Lesjean et al., 2002) while maintaining a lower shear stress than an external MBR.

A review of relevant literature revealed few studies on the determination of kinetic constants for EE2 removal by AS (De Gusseme et al., 2009; Shi et al., 2004; Vader et al., 2000). Therefore, the aim of this research was to determine kinetic parameters for EE2 sorption and biodegradation by nitrifying AS. Biodegradation of the synthetic hormone was previously studied with concentrations between 1 and 1 $\mu g_{EE2}$ $L^{-1}$ (Ternes et al., 1999). However, no significant decrease in EE2 concentrations was detected. Thus, experiments were performed with higher values, in the $mg_{EE2}$ $L^{-1}$ range, to quantify both phenomena (sorption and biodegradation) involved in EE2 removal. To increase nitrifying activity, an external-immersed MBR was used with a specific influent and without any sludge waste to increase SRT and thus enhance autotrophic biomass growth.

## 2. Methods

### 2.1. Chemicals and solvents

Carbon source and nutrients ($C_6H_{12}O_6$, $(NH_4)_2SO_4$, $NaHCO_3$, $KH_2PO_4$, $MgSO_4$, and $CaCl_2$) were supplied by Chem-Lab (>99%, Zedlgem, Belgium). The reagent 1-allyl-2-thiourea (98%) was purchased from Aldrich (L'Isle d'Abeau, France) and sodium azide (99%) was purchased from Acros organics (Noisy-le-Grand, France). 17α-ethynylestradiol (⩾98%) and β-estradiol-17-acetate (⩾99.9%) were obtained from Sigma–Aldrich (L'Isle d'Abeau, France). β-Estradiol-17-acetate (⩾99.9%) was used as an internal standard. EE2 stock solutions were prepared in absolute ethanol (⩾99.8%, ACS reagent grade) from Carlo Erba (Val de Reuil, France). Distilled water was used for the analytical method. HPLC-grade methanol (⩾99.9%) was supplied by Sigma. Reagent kits used for spectrophotometric ammonium and nitrate analyzes were obtained from Merk (Darmstadt, Germany).

### 2.2. The MBR system

The lab-scale MBR was composed of a 60-L bioreactor and polysulfone membranes submerged in a 250-mL external carter (130 hollow fibres, molecular weight cut-off 0.2 μm, total surface 0.2 $m^2$, Polymem, France). Peristaltic pumps were used for the synthetic influent, permeate and sludge recycle back to the membrane module. Membrane fouling was limited by air sparging (6 L $min^{-1}$) in the external carter. The MBR was operated at a hydraulic retention time (HRT) of 50 h without wasting any sludge to maximize the SRT. The pH was maintained constant at seven by an automatic controller with a sodium bicarbonate ($NaHCO_3$) solution (60 g $L^{-1}$).

### 2.3. Acclimation of autotrophic biomass

The lab-scale MBR was started with activated sludge (AS) collected from a municipal WWTP operated with nitrification and denitrification tanks (165,000 population equivalent, Aix-en-Provence, France). The AS was concentrated in the MBR until 11 g $L^{-1}$ of mixed liquor volatile suspended solids (MLVSS) and a final volume of 50 L were achieved. The synthetic influent was prepared without any organic carbon source and with mass ratios of 1 $(NH_4)_2SO_4$, 0.4 $NaHCO_3$, 0.2 $KH_2PO_4$, 0.1 $MgSO_4$, and 0.02 $CaCl_2$. The ammonium load was increased from 0.02 to 0.16 $kg_{N-NH_4}$ $kg^{-1}_{MLVSS}$ $d^{-1}$ to enhance the growth of autotrophic micro-organisms.

### 2.4. Analytical methods

Mixed liquor suspended solids (MLSS) were determined from AS centrifugation (15,892g, 30 min) followed by 24-h drying at 105 °C. MLVSS were then determined from heating the MLSS at 550 °C for 2 h. Ammonium, nitrate and polysaccharide (PS) were analyzed in the supernatant by spectrophotometry (Spectro Aquamate, Thermo spectronic, Cambridge, UK) with reagent kits for ammonium (2–150 mg $L^{-1}$ $N–NH_4$, R.S.D. 2%) and nitrate (0–20 mg $L^{-1}$ $N–NO_3$, R.S.D. 3%) and based on the method of Dubois et al. (1956) for PS (R.S.D. 9%).

### 2.5. Biological methods

Ammonium removal rates were determined during the acclimation period from 4-h batch experiments. Aerated reactors were filled with 1 L of the acclimated AS and 0.02 $g_{N-NH_4}$ $g^{-1}_{MLVSS}$ was added. The ammonium concentration was analyzed 4 h after nutrient addition. Additionally, respirometry tests were used to differentiate autotrophic and heterotrophic micro-organisms. An aerated reactor was filled with 1 L of the acclimated AS and every 2 min, 50 mL was sampled and injected into another reactor without oxygenation to measure the oxygen uptake rate (OUR) with a continuous dissolved oxygen probe (HQ 40d, Hach LDO, Düsseldorf, Germany). Then, specific nutrients or inhibitor were successively added to the aerated reactor.

The respirometry method was divided into four steps. Endogenous respirations of autotrophic and heterotrophic micro-organisms were first measured over a period of 1 h. Secondly, ammonium was added (0.02 $g_{N-NH_4}$ $g^{-1}_{MLVSS}$) to measure the maximum activity of autotrophic micro-organisms (ammonium removal was confirmed to be correlated with nitrate production). Thirdly, autotrophic micro-organisms were inhibited with allylthiourea (inhibitor of the nitrifying enzyme AMO) to isolate heterotrophic endogenous respiration. A concentration of 0.1 $g_{allylthiourea}$ $g^{-1}_{MLVSS}$ was shown to inhibit the respirometry activity, and nitrates were not produced after ammonium addition (0.02 $g_{N-NH_4}$ $g^{-1}_{MLVSS}$), which validated the autotrophic inhibition. Lastly, maximum activity of heterotrophic micro-organisms was quantified after glucose addition (equivalent to 0.3 $g_{COD}$ $g^{-1}_{MLVSS}$).

### 2.6. EE2 removal in the MBR

After 53 days of acclimation, EE2 was added into the synthetic MBR influent to reach a final concentration of 1 $mg_{EE2}$ $L^{-1}$. EE2 concentrations in the permeate were followed for 60 h. The bioreactor volume was reduced to 10 L, and the HRT was reduced to 19 h. The MLVSS concentration was verified to be constant at 7 g $L^{-1}$. The same continuous MBR operation and addition of EE2 was performed with AS directly sampled from the WWTP.