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Removal performance of nitrogen and endocrine-disrupting pesticides simultaneously in the enhanced biofilm system for polluted source water pretreatment

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HIGHLIGHTS

- No significant change of EDPs removal with increase of NH_4^+–N and NO_3^-–N transfer.
- Simultaneous denitrification and EDPs removal in anoxic niches via reed addition.
- Many species related to the nitrogen and EDPs removal enhanced with reed addition.

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ABSTRACT

The removal performances of nitrogen and trace levels of endocrine-disrupting pesticides (cypermethrin and chlorpyrifos) were studied in the enhanced biofilm pretreatment system at various substrates concentrations and dissolve oxygen (DO) niches. No significant change of EDPs removal occurred with the increased feed of ammonia nitrogen in aerobic batch tests or nitrate in anaerobic batch reactors, but significantly enhanced via reed addition both in aerobic and anaerobic conditions. Simultaneously enhanced denitrification and EDPs removal were achieved in the anoxic niche with reed addition. The results of denaturing gradient gel electrophoresis (DGGE) indicated that new bands appeared, and some bands became more intense with the reed addition. Sequences analysis showed that the dominant species belonged to *Methylophilaceae*, *Hyphomicrobium*, *Bacillus* and *Thauera*, which were related to the nitrogen or EDPs removals. In addition, the growth of functional heterotrophic microbes may be promoted via reed addition.

1. Introduction

The presence of endocrine disrupting compounds (EDCs) in natural waters has the ability to mimic or inhibit the natural action of the endocrine systems in invertebrates and possibly human beings at trace levels ($\mu g\ l^{-1}$ or $ng\ l^{-1}$) (Frye et al., 2012; Hirai et al., 2006; Pawlowski et al., 2004; Silva et al., 2012). EDCs have become a key priority in water quality control over last decade, which has been responded by wide investigations of EDCs removal in wastewater and water treatment works all over the world (Kim et al., 2007; Stasinakis et al., 2008). It is now known that removal efficiencies of EDCs can greatly vary in various processes. Currently biological processes are still popular approaches due to its low maintenance cost and effective contaminants removal.

In biological systems, generally aerobic condition favors EDCs removal as compared with that in anaerobic condition (Xue et al., 2010). There may be two reasons for this: one was that most of heterotrophic bacteria that can degrade EDCs adapted to aerobic environment; the other was metabolic nitrifiers due to the enzymatic action of ammonia monooxygenase (AMO) in aerobic conditions (Forrez et al., 2009). Yi and Harper (2007) proved that there was a positively linear relationship between17α-ethynylestradiol reduce rate and the ammonia biotransformation rate. However, EDCs and initial ammonia concentrations in most previous studies were much higher than those in effluent of wastewater treatment plant or natural waters. Gaulke et al. (2008) reported that 17α-ethynylestradiol removal at low concentrations in activated sludge systems was not attributed to cometabolic degradation, but most possibly due to the effect of heterotrophic bacteria (Gaulke et al., 2008). Still, the information of relationship between EDCs removal

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at trace level and nitrogen removal at low concentration is still limited.

No matter the effects of heterotrophic bacteria or the cometabolic degradation by AMO that mainly cause the EDCs removal, it is known that microbial population densities and community structure are the key factors for determining the performances of biological systems. Effects of microbial population densities (MLVSS) on EDC of 17β-estradiol removal was reported by Li et al. (2005), which suggested that higher MLVSS was advantage to the increasing in 17β-estradiol degradation rate (Silva et al., 2012). Thus, it is essential to develop ways to improve bacteria growth. In addition, environmental conditions (e.g. pH, temperature and dissolve oxygen), nutrients level (e.g. carbon, nitrogen and phosphorus) could also significantly affect toxic organics removal, especially for water treatment in oligotrophic environment.

Phosphorus addition in drinking water showed that the relative abundance of perchlorate-reducing bacteria (PRB) *Dechloromonas* and *Azospira* in the bench-scale reactor increased from 15.2% and 0.6% to 54.2% and 11.7%, respectively (Yoon et al., 2006). Studies on nutrients limiting biofilm development in water treatment process and distribution systems demonstrated a positive relationship between biodegradable carbon source in water and bacterial growth in biofilm (Chandy and Angles, 2001; Vanderkooij, 1992). Pure cultures studies also showed that degradation ability of bacteria was positively influenced by the presence of supplementary carbon sources and other nutrients (Cycon et al., 2009; Watanabe et al., 2012). For example, the degradation of beta-cypermethrin (endocrine-disrupting pesticide) significantly enhanced at the presence of glucose, beef extract and yeast extract, the degradation efficiencies reached to 85.4%, 90.4% and 87.0%, respectively (Chen et al., 2011); it was also reported that the initial degradation rate of bisphenol-A via *Pseudomonas monteilii* strain N-502 was obviously accelerated with the addition of Ca^{2+}, Mg^{2+} and folic acid (Masuda et al., 2007).

Since the molar ratio of carbon, nitrogen to phosphorus required for bacterial growth is approximately 100:10:1, carbon is the most needed for bacterial growth but always limited in oligotrophic waters. Furthermore, carbon source is also a good electron donor for denitrification. For stimulating the growth of microorganism and promoting the performance, extra carbon source were often used in many studies (Ovez et al., 2006; Park and Yoo, 2009; Warneke et al., 2011). The extra carbon sources often used were soluble carbon source (such as methanol, ethanol, acetic acid, acetate, etc.) and solid carbon source (such as wheat straw, rice husk, reed, *Typha latifolia*, *Elodea canadensis*, etc.) (Ovez et al., 2006; Park and Yoo, 2009; Warneke et al., 2011). Previous studies showed that solid carbon source was much cheaper than soluble carbon source (Boley et al., 2000; Soares, 2000). The reed as a representative solid carbon source used in this study, depending on its characteristics of cost-saving, widely distributed and abundant carbon content (Ovez et al., 2006).

Up to now, previous studies have reported that nitrate and endocrine-disrupting pesticides (EDPs) could be simultaneously removed via external carbon source addition, e.g. Ethanol (Choi et al., 2006), biodegradable polymer poly (Ginige et al., 2004), wheat straw (Xu et al., 2012) and so on. However, the nitrogen and EDPs in these reported studies were much higher than those occurrences in oligotrophic niche, where it is hard to realize the simultaneous removal of nitrogen and EDPs. What is more, little studies have been focused on the characteristics of bacteria community in simultaneous denitrification and EDPs removal, especially in source water pretreatment systems.

Due to the information on the removal of trace EDCs in oligotrophic waters remains limited, the effects of initial ammonia, nitrate and carbon source feeding on the removal performances of selected endocrine-disrupting pesticides (cypermethrin and chlorpyrifos) were studied in biofilm source water pretreatment system. The aim of this study was to (1) ascertain whether EDPs cometabolic degradation occurred at trace levels by ammonia oxidization effects in oligotrophic environment; (2) examine the fate of cypermethrin and chlorpyrifos at presence of various nitrogen and carbon source.

2. Methods

2.1. Reagents

Two EDPs (chlorpyrifos and cypermethrin) were purchased from national standard research center in China and used without further purification; the detail description is shown in Table 1. The inorganic salts used in the synthetic water were NH_4Cl, KNO_3, KH_2PO_4, and methanol was used as carbon source. All reagents for solid phase extraction (SPE) and gas chromatography mass analyses were of HPLC grade. Reed leachate was self-made in laboratory. Firstly the wilt reed used was collected from a wetland nearby Zhejiang University in China, which was treated by a cryogenic impact grinder. The fine powder of reed was emerged in purified water and heated at 120 °C for 1 h. The supernatant was collected as reed nutrition, and stored at 4 °C for use. The nutrient components of reed nutrition, such as total organic carbon (TOC), ammonia, nitrite, nitrate, phosphate, total nitrogen and total phosphorus were analyzed (Table 2).

2.2. Experiments design

To study the relationship between EDPs and nitrogen removal in oligotrophic environment, the initial ammonia in aerobic reactors (OB1–OB3) and nitrate in anaerobic reactors (AB1–AB3) were both conducted in different levels; the effect of reed addition on EDPs and nitrogen removal were also studied. The detail operation parameters are presented in Table 3. All the experiments were conducted in plexiglass batch reactors with working volume of 4.2 L. There were double for each operation condition. TA-II elastic filler, purchased from Tianyu Environmental Protection Engineering Co., Ltd. (Hangzhou, China), was used as biofilm carrier, and all the batch reactors were filled with the same volumetric carriers. This kind of carrier had a diameter and surface area of 200 mm and 200–300 $m^2\ m^{-3}$, respectively. The DO in aerobic and anaerobic reactors was at the range of 7.5–8.6 mg l^{-1} and 0.01–0.45 mg l^{-1}, respectively. The other operational parameters were shown as follows: water temperature of (25 ± 2) °C, hydraulic retention time (HRT) of 24 h, pH 7.2–7.8. After the steady operation performance was achieved, regular index were analyzed every other day and EDPs were analyzed every week.

2.3. Analytical methods

2.3.1. EDPs analysis

Chlorpyrifos and cypermethrin analysis were carried out by gas chromatography tandem mass spectrometer (GC–MS) after solid phase extraction (SPE). Prior to SPE, water sample was filtered through Whatman GF/F glass microfiber membrane filters with pore diameter of 0.45 μm. Conditioning of the ENVI-18 cartridges (Supelclean, 6 cc, 500 mg) with 6 ml dichloromethane followed by 6 ml methanol and then washed with 12 ml pure water. A total of 500 ml water sample was passed through cartridge at the flow rate of 5 ml min^{-1}. Then the cartridges were washed with 5 ml pure water and dried under vacuum for 0.5 h. The EDPs extracts were eluted with 20 ml of dichloromethane and tenderly dried under nitrogen gas (Organomation nitrogen evaporators, USA).