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Acute and chronic responses of denitrifying culture to diclofenac

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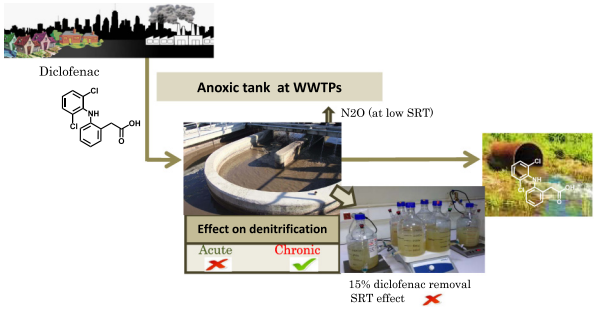
HIGHLIGHTS

- Continuous diclofenac amendment resulted in a decrease in the gas production.
- Diclofenac removal in diclofenac-acclimated culture was less than 15%.
- N_2O reduction was negatively affected in the presence of diclofenac at low SRT.
- Diclofenac resulted in a decrease in nitrate removal rate especially at low SRT.

GRAPHICAL ABSTRACT



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ABSTRACT

Acute and chronic effect as well as biodegradation potential at different sludge retention times (SRTs) of a priority pollutant, diclofenac on denitrification process was assessed. The continuous amendment of the culture for 6 months with 1 μg/L diclofenac resulted in 30% decrease in gas production. The average diclofenac removal observed in the diclofenac-acclimated culture was less than 15%. Batch tests showed that nitrate was removed in diclofenac free-control reactor at a higher rate compared to diclofenac amended reactor. Although, SRT did not have any progressive effect on diclofenac degradation, the system operated at low SRT was more sensitive to diclofenac and resulted in an increase in N_2O emission. Wastewater treatment plants (WWTPs) operated at higher SRTs may tolerate and recover from the adverse effects of such micropollutants. The study can lead to other researchers to understand the fate and effect of other emerging pollutants in the anoxic unit of WWTPs.

1. Introduction

In the last decades, high amounts of pharmaceuticals have been found in environmental matrices like surface water, ground water, soil and sediments. Although their concentrations are measured in the range of ng/L to μg/L in aquatic environments (Zhang et al., 2008; Aydin and Talinli, 2013); they are still a cause of concern because of their potential chronic effects and synergic action of their mixtures not only on aquatic life but also on human health. Among these pharmaceuticals, diclofenac is a frequently used non-steroidal anti-inflammatory drug (NSAID) which is detected in a wide variety of environmental matrices that include surface water (Carmona et al., 2014) and groundwater (Félix-Canedo et al., 2013). The globally consumed amount of diclofenac has been reported as 940 tons per year (Zhang et al., 2008). NSAIDs were mostly observed in the influents of municipal WWTPs and pharmaceutical manufacture WWTPs (Sim et al., 2011; Yannik et al., 2012). Diclofenac is one of the most frequently detected pharmaceutical in the effluents of municipal WWTPs (Verlicchi et al., 2012). The concentrations of diclofenac in municipal wastewaters were reported in the range of 0.44 and 7.1 μg/L with mean concentrations of 0.11 and 2.3 μg/L (Vieno and Sillanpää, 2014). Concentrations of diclofenac determined in the effluent of conventional WWTPs are >1 μg/L (Vieno and Sillanpää, 2014). Andreozzi et al. (2003) reported that diclofenac has been detected in different

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European WWTPs' effluents up to 5.5 μg/L concentration. The pharmaceutical manufacture WWTP influents showed relatively high levels of pharmaceuticals attributed to the production processes of pharmaceuticals (Larsson et al., 2007). Maximum concentration of diclofenac in pharmaceutical manufacturer's wastewater was reported as 203 μg/L; significantly higher than normally detected concentration in municipal wastewater (Sim et al., 2011). The presence of diclofenac in the effluents of these WWTPs may pose a threat to aquatic life and may re-enter the water cycle when discharged to surface water without sufficient treatment. Zhang et al. (2008) reported that ecotoxicological studies on diclofenac showed no acute toxicity effects at their environmental concentrations but they indicated the requirement for cautious attention in terms of chronic effects. Failure can occur easily in WWTPs especially due to the slow-growing and sensitive characteristics of both nitrifying and denitrifying microorganisms. Additionally, changes in microbial communities under a selection pressure caused by a low, but chronic exposure to pharmaceuticals may have potential adverse effects also on the receiving ecosystems (Corcoll et al., 2014). Therefore, in addition to the studies that should be performed to assess the acute effect of pharmaceuticals in WWTPs and in natural environments, the chronic effect of lower concentrations (i.e. 1 μg/L) should be also investigated.

Conventional wastewater treatment processes (i.e. activated sludge systems) in WWTPs have limited micropollutant elimination capacities as they are not specifically designed to remove pharmaceuticals from wastewater (Joss et al., 2006; Sari et al., 2014). The process design and operational conditions are the factors that greatly affect the performance of the WWTPs regarding diclofenac removal (Luo et al., 2014). Among the conventional activated sludge processes; A/O and A2O systems are widely applied for nutrient removal. In this context, denitrification is an important process in WWTPs to achieve effective nitrogen removal from wastewater. In the literature, the number of studies reflecting the fate of micropollutants under anoxic conditions is limited. In general, biodegradation of organic compounds under anoxic conditions proceeds slower than under aerobic conditions (Schuman, 2008; Kujawa-Roeleveld and Schuman, 2008). Some of the previous studies have reported the suitability of anoxic environments for micropollutant removal. Stasinakis et al. (2009) observed better removal of diuron during batch tests under anoxic conditions (>95%) in comparison to that in aerobic conditions (60%). Studies on the biodegradation potential of two pharmaceuticals namely sulfamethoxazole and carbamazepine under both anoxic and aerobic conditions showed that carbamazepine was only removed under aerobic conditions, while the biodegradation of sulfamethoxazole occurred under both anoxic and aerobic conditions (Hai et al., 2011). Zwiener and Frimmel (2003) compared short-term biodegradation of clofibric acid, ibuprofen, and diclofenac in oxic and anoxic biofilm reactors. The anoxic biofilm reactor achieved much lower removal of ibuprofen (17–21%) and higher removal of diclofenac (34–38%) and clofibric acid (26–30%). Some persistent substances (i.e. diclofenac, sulfamethoxazole, trimethoprim, and carbamazepine) were removed at a limited level (25%) by biological treatment with either nitrifying (oxic) or denitrifying (anoxic) bacteria (Suarez et al., 2010; Luo et al., 2014).

The objectives of this study are to (a) investigate the chronic inhibitory effect and biodegradation potential of diclofenac under denitrifying conditions at a relevant concentration observed in most municipal WWTPs' influents; (b) understand the acute inhibitory effect and biodegradation potential of diclofenac at relatively higher concentrations that can be observed in pharmaceutical manufacturer's WWTPs and under unpredicted conditions in municipal WWTPs; and (c) identify the effect of sludge retention time (SRT) on biodegradation potential of diclofenac.

2. Methods

2.1. Culture development

2.1.1. Assessment of acute and chronic effect of diclofenac

Two denitrifying enrichment cultures were developed from anoxic sludge of a municipal WWTP located in Istanbul, Turkey (Table 1). Two cultures (referred to diclofenac-free control and diclofenac-acclimated) were initiated by diluting 200 mL of sludge sample with mineral media (Okutman Tas and Pavlostathis, 2010) up to 2 L in helium-flushed, 3 L glass flask reactors, capped with Teflon-lined stoppers and operated as semi-continuous reactors. The glass reactors were modified to have a gas sampling port sealed with Teflon stopper and aluminum crimp caps at the top and a liquid sampling valve (gastight glass and Teflon sampling port) at the bottom. The outlet of the valve was fitted with a short piece of Teflon tubing and stainless steel luer hub (Cole-Parmer) to connect syringes to the reactor for any additions and sampling withdrawals. Both cultures were fed at the beginning of each 7-day feeding cycle with glucose and yeast extract resulting in initial concentrations of 300 and 15 mg/L, respectively. 350 mL of the culture was wasted from the complete mixture and then filled with fresh anoxic media for every feeding cycle (7-day) to maintain 40 days SRT. Diclofenac-acclimated culture was amended with 10 μg/L diclofenac (98%; CAS 15307-79-6) dissolved in methanol for once at the startup of the reactor and then for the following feeding cycles diclofenac concentration decreased to 1 μg/L which is a relevant concentration observed in the influents of WWTPs (Sari et al., 2014; Vieno and Sillanpää, 2014), whereas diclofenac-free control culture was fed with pure methanol (40 μL). The nitrate concentration was kept in excess (>100 mg N/L) in both cultures not to shift to methanogenic conditions. Both reactors were operated in a 22 °C constant temperature room.

2.1.2. Assessment of sludge retention time effect on diclofenac removal efficiency

Six denitrifying semi-continuous reactors were set-up with the inoculum taken from the anoxic unit of a municipal WWTP located in Istanbul, Turkey (Table 1) in order to investigate the effect of SRT on diclofenac removal under anoxic conditions. In this context, three different SRTs (10, 20, and 40 days) were tested with the reactors operated as diclofenac-free control and diclofenac-acclimated. Anoxic cultures were initiated by diluting 1 L of sludge sample with 1 L of mineral media (Okutman Tas and Pavlostathis, 2010) in helium-flushed, 3 L glass flask reactors, capped with Teflon-lined stoppers. All reactors were operated at constant room temperature (22 °C) and mixing was provided with magnetic stirrers. All cultures were fed twice in a week with glucose, and yeast extract resulting in the initial concentrations of 300 mg/L, and 15 mg/L for 3-days feeding cycle and 400 mg/L, and 20 mg/L for 4-days feeding cycle, respectively. The diclofenac-acclimated cultures were amended with 1 μg/L diclofenac dissolved in methanol, whereas the diclofenac-free control cultures were fed with pure methanol (40 μL). The nitrate concentration was kept in excess (>100 mg N/L) in all reactors. Reactors were filled with fresh anoxic media up to 2 L after withdrawing the sludge in order to provide the required SRT.

2.2. Batch assays

2.2.1. Effect of initial diclofenac concentration on denitrification

The acute effect of initial diclofenac concentration on the denitrifying cultures was investigated in a batch assay using triplicate 245 mL serum bottles (effective volumes of 180 mL each) which were sealed with Teflon-lined septa and flushed with helium gas.