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# Effects of selected pharmaceuticals on nitrogen and phosphorus removal bioprocesses

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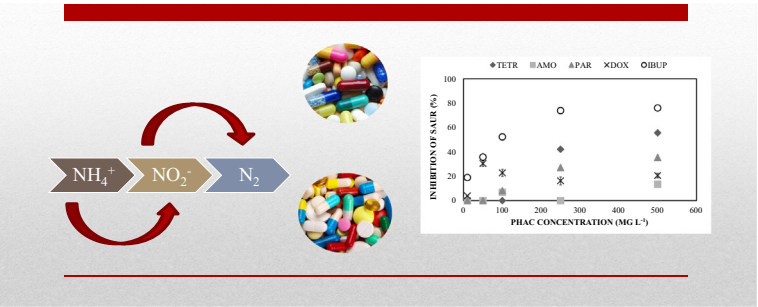
## HIGHLIGHTS

- The anoxic P uptake via nitrite was more tolerant to PhaCs than the aerobic one.
- IBUP was the most toxic single tested compound to nitrogen removal via nitrite.
- Denitritation and aerobic P uptake were the most sensitive processes.

## GRAPHICAL ABSTRACT

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## ABSTRACT

The inhibitory effect of five selected pharmaceuticals on the nitrogen and phosphorus removal bioprocesses was investigated with emphasis on the via nitrite removal pathway. Biomass was collected from a sequencing batch reactor (SBR) and was spiked with specific pharmaceutical concentrations (10–500 mg $L^{-1}$). Batch activity tests were conducted to determine the level of inhibition caused by the selected pharmaceuticals and their combinations to the rate of nitritation, denitritation, phosphorus uptake via nitrite and via oxygen and phosphorus release. The highest inhibition of nitritation was observed when 250 mg $L^{-1}$ of ibuprofen mg $L^{-1}$ and 250 of paracetamol were added. Out of the 12 different combination that were tested, sPRR was the bioprocess that was least inhibited by the pharmaceuticals in 4 cases ($p < 0.05$), while the aerobic phosphorus uptake was the most severely inhibited bioprocess in 4 cases ($p < 0.05$).

*Abbreviations:* AMO, ammonia monooxygenase; AMO, amoxicillin; Anammox, anoxic ammonium oxidation; AOB, ammonium oxidizing bacteria; Bet42a, *Betaproteobacteria*; CG, conventional granules; COD/N, chemical oxygen demand/nitrogen; COD, chemical oxygen demand; Cte, *Comamonas* sp., *Acidovorax* sp., *Hydrogenophaga* sp., *Aquaspirillum* sp.; DO, dissolved oxygen; DOX, doxycycline; DPAOs, denitrifying phosphorus accumulating organisms; DPRN, denitrifying phosphorus removal via nitrite; FISH, fluorescence *in situ* hybridization; FL, fermentation liquid; HRT, hydraulic retention time; IBUP, ibuprofen; $IC_{50}$, half maximal inhibitory concentration; MZ1, *Thauera* sp; NG, nitrifying granules; $NH_4$-N, ammonium-N; NLR, nitrogen loading rate; $NO_2$-N, nitrite-N; $NO_3$-N, nitrate-N; NOB, nitrite oxidizing bacteria; $NO_x$-N, nitrate-N and nitrite-N; NSAID, non-steroidal anti-inflammatory drug; Nsm156, *Nitrosomonas* spp., *Nitrosococcus mobilis*; NSO1225, ammonio-oxidizing-β-*Proteobacteria*; OFMSW, organic fraction of municipal solid waste; OLR, organic loading rate; OTC, oxytetracycline; OUR, oxygen uptake rate; $OUR_{max}$, maximum oxygen uptake rate; Nsm156, *Nitrosomonas* spp., *Nitrosococcus mobilis*; PAOs, phosphorus accumulating organisms (*Accumulibacter phosphatis*); PAR, paracetamol; PhaC, pharmaceutical; $PO_4$-P, phosphate-P; sAUR, specific ammonium uptake rate; SBR, sequencing batch reactor; sNUR, specific nitrite uptake rate; sOUR, specific oxygen uptake rate; $sOUR_{max}$, maximum specific oxygen uptake rate; sPRR, specific phosphorus release rate; $sPUR_{aer}$, specific phosphorus uptake rate under aerobic conditions; $sPUR_{anox}$, specific phosphorus uptake rate under anoxic conditions; SRT, solids retention time; TETR, tetracycline; UASB, upflow anaerobic sludge blanket; vNLR, volumetric nitrogen loading rate; VSS, volatile suspended solids; ZRA23a, *Zoogloea lineage, not Z. Resiniphila*; WWTPs, wastewater treatment plants.

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In most cases, anoxic phosphorus uptake via nitrite was more tolerant compared to aerobic phosphorus uptake. Thus, its implementation is more favorable for the treatment of effluents with high pharmaceutical concentrations.



## 1. Introduction

Pharmaceuticals (PhaCs) are widely used to protect health and prevent/control diseases in both humans and animals [1]. Paracetamol (PAR), tetracycline (TETR), doxycycline (DOX) and amoxicillin (AMO) are extensively dosed to animals in livestock farms to prevent and treat diseases and for weight promotion of animals. A significant percentage of the substances used in farms, are not metabolized by the animals and are excreted into the environment in active forms. When treated animal wastes are applied as supplements to fertilizers they can find their way into the receiving environment and can be present either as metabolite or as the parent compound [2]. Ibuprofen (IBUP) is a non-steroidal anti-inflammatory drug (NSAID) that is used to relieve pain and reduce fever. Its extensive use by humans and animals renders its investigation important [3].

In municipal wastewater treatment plants (WWTPs), pharmaceuticals are usually present in trace levels of the order to ng $L^{-1}$ and thus have a minor impact on the bacterial activity. Biological degradation of pharmaceuticals within municipal WWTPs occurs. Joss et al. [9] concluded that the current biological wastewater treatment schemes are not effective in removing pharmaceuticals. In particular, only 4 out of 35 compounds were degraded by more than 90%, while 17 compounds were removed by less than 50% [9].

The concentrations of antibiotics in hospital and pharmaceutical effluents from industries can reach 100–500 mg $L^{-1}$ [4]. Significant concentrations of TETR (100–1000 mg $L^{-1}$) [5], DOX (0.5–16 mg $L^{-1}$), and AMO (0.5–64 mg $L^{-1}$) [6] have been found in livestock effluents. As a result, the anaerobic effluents of livestock waste contain significant pharmaceutical concentrations. Considering an approximate pig weight of 100 kg and the consumption of TETR in pig farms to be 40 mg $kg^{-1}$ live weight $d^{-1}$ during 10 d it results in 1.5–6 gTETR per pig per day [7]. Considering that the pig's daily excreta is approximately 15 L and that TETR is metabolized in the animal by 20–30%, the TERT concentration in the livestock effluents can be in the range of 70–320 mg $L^{-1}$. Assuming that the anaerobic digestion process degrades TERT by 70% [8], the TETR concentration in the anaerobic effluents ranges from 20 to 100 mg $L^{-1}$.

Several studies have determined the effect of the pharmaceutical veterinary products on the anaerobic digestion of manure [8,10]; little information is available concerning the effect of veterinary pharmaceuticals on nitritation/denitritation and phosphorus removal processes via nitrite.

In the nitritation/denitritation biological process, ammonium is oxidized to nitrite and is subsequently reduced to gaseous nitrogen. Therefore, there is a short-cut in the nitrogen removal process, since the oxidation of nitrite to nitrate is by-passed. To accomplish nitrogen removal via nitrite, it is necessary to inhibit or washout the nitrite oxidizing bacteria (NOB) and to promote the development of ammonium oxidizing bacteria (AOB). The merits of these advanced processes (i.e. lower organic carbon source requirements for denitritation and aeration requirements for nitritation compared to conventional nitrification/denitrification) make them very promising for the treatment of the anaerobic supernatant of the digestate [11]. Significant cost savings and carbon footprint reduction can arise when liquid effluent derived from the organic waste is supplied as carbon source in the nitrogen and phosphorus removal process via the nitrite pathway [12,13].

Some literature is available on antibiotic inhibition of nitritation and anammox [14,15]. Antibacterial agents, such as TETR and olaquindox, exert an inhibitory effect on Gram-negative bacteria [16]. During the anaerobic digestion, *bacteroides* spp. and *Clostridium* spp. bacteria are inactivated by the presence of AMO [17]. In the case of oxytetracyclines, an $IC_{50}$ level of ~500 mg $L^{-1}$ was determined for anammox bacteria [18]. During the long-term operation of an SBR anammox system for the treatment of wastewater produced from the manufacturing of colistin sulfate and kitasamycin, the accumulation of toxic compounds resulted in the failure of the anammox process [14]. Nitrification has been found to be more sensitive to the presence of TETR than the other activated sludge processes, with nitrite oxidation being completely inhibited at TETR concentrations of 200 mg $L^{-1}$, while the impact on heterotrophic bacterial growth was low [16,19]. Very recently, Alvarino et al. [20] compared the inhibitory effect of DOX and PAR on anammox and nitritation/denitritation and concluded that anammox bacteria were more sensitive to these products. However, all short-cut nitrogen and phosphorus removal biological processes were inhibited to some extent at DOX and PAR concentrations $\geqslant$250 mg $L^{-1}$. Denitrifying phosphorus removal by phosphorus accumulating organisms (PAOs) that can utilize nitrate or nitrite as electron acceptors has recently gained attention [21]. It is therefore important to document the inhibition of denitrifying PAOs caused by the presence of different pharmaceuticals. Several studies have examined the effect of pharmaceuticals on the activity of biomass in the conventional nitrification/denitrification. Acute inhibition experiments and long-term inhibition tests indicated that antibiotics mainly reduced the nitrification rate, with the exception of a few studies reporting no appreciable effect or even stimulatory effects [22,23]. However, the published works are limited to three kind of antibiotics; chlor-amphenicol, b-lactams and TETR [24]. It is important to evaluate the effects of target pharmaceuticals on the activity of autotrophic and heterotrophic biomass in nitritation/denitritation and the denitrifying phosphorus removal via nitrite for the treatment of anaerobic effluents.

The aim of this work is to evaluate the inhibitory effect of selected pharmaceuticals on the nutrient removal processes via nitrite. The increasing application of the via nitrite bioprocesses for the treatment of livestock effluents creates the need to examine the effect of pharmaceuticals that can be found in these type of streams.

## 2. Materials and methods

### 2.1. Chemicals

The pharmaceutical substances used in this work were paracetamol (PAR) (CAS No. 103-90-2, $CH_3CONHC_6H_4OH$, MW is 151.16), doxycycline (DOX) (CAS No. 10592-13-9 $C_{22}H_{24}N_2O_8 \cdot HCl$, MW is 480.90), tetracycline (TETR) (CAS No. 64-75-5 $C_{22}H_{24}N_2O_8 \cdot HCl$, MW is 480.90) ibuprofen (IBUP) (CAS No. 15687-27-1 $C_{13}H_{18}O_2$ MW 206.28) and amoxicillin (AMO) (CAS No. 26787-78-0 $C_{16}H_{19}N_3O_5S$, MW is 365.4) of Sigma Aldrich (purity 98–100%). The chemicals used for the batch experiments were sodium