Removal mechanisms of emerging micropollutants in an innovative low environmental footprint wastewater technology: the SIAL process

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Abstract New designs for a more sustainable treatment of sewage are including the addition of an anaerobic step in the water line. This study presents the SIAL process which combines an anaerobic UASB reactor with an anoxic-aerobic stage, where the dissolved methane present in the UASB effluent is used as organic carbon source for N removal. The removal of 11 organic micropollutants (OMPs) was assessed. The reactor was operated in two periods: I) attached and suspended biomass and II) only attached biomass in the anoxic-aerobic stage. The best results in terms of N removal were achieved in P1 (24.7 ± 5.9 mg NT L^{-1}), while in P2 the N removal decreased (17.2 ± 5.4 mg NT L^{-1}). This was mainly attributed to the nitrogen species present: nitrite during period 1 and nitrate during period 2.

The removal of OMPs was dependent on the redox conditions. Sulfamethoxazole, trimethoprim, and naproxen were readily biodegraded in the anaerobic step, whereas ibuprofen or bisphenol A were mainly removed under aerobic conditions. These results indicate the positive effect related to the combination of different redox conditions as a technological strategy to enhance the removal of OMPs. Evidence of the cometabolic biotransformation of certain OMPs has been found such as the influence of nitrification activity on the removal of bisphenol A.

Keywords: Organic micropollutants, N removal, sewage, redox conditions, methane emissions.

1. Introduction

One of the key issues in wastewater treatment is the operational cost related to energy consumption. With the aim of reducing this important contribution to the overall operational cost, innovative approaches are under development. In this sense, an anaerobic methanogenic step in the water line would allow the reduction of the aeration costs. Additionally, the reduction of organic matter to methane instead of carbon dioxide represents a valorization of this residue in terms of energy production. This approach has been employed in warm countries and even in those with temperate or mild climate. However, the fraction of the methane that remains dissolved in the liquid phase (and thus useless for biogas recovery) can represent up 25-50%, especially high at ambient temperatures below 20ºC (Noyola *et al*., 2016). In fact, these liquid effluents from anaerobic digesters are responsible of about 2% of the total emissions of this greenhouse gas (Metz *et al*., 2007). Although aerobic treatment is the common post-treatment alternative for these streams, part of the dissolved methane is not effectively removed, just released into the atmosphere by stripping.

The use of the classical anoxic-aerobic configuration for the simultaneous removal of COD and nutrients as a post-treatment is often limited by the low COD content present after the anaerobic stage, which limits denitrification. New consortia of bacteria/archaea (methanotrophs) able to use methane as a carbon source for denitrification have been discovered recently, which can use both nitrate or nitrite (Ettwig *et al*., 2010). The pathway is a function of the dissolved oxygen concentration. Aerobic methanotrophs are able to convert methane into oxidized species that can be employed as an organic carbon source by the denitrifying heterotrophs leading to the following overall stoichiometric reaction (Zhu *et al*., 2016).

$$5\,CH_4 + 5\,O_2 + 4\,NO_3^- + 4\,H^+ \rightarrow 2\,N_2 + 12\,H_2O + 5\,CO_2$$

The denitrification coupled to anaerobic methane oxidation (DAMO) is carried out by the DAMO bacteria (*Candidatus Methylomirabilis oxyfera*) or DAMO archaea (*Candidatus Methanoperedens nitroreducens*) with nitrite or nitrate as electron acceptors, respectively (Modin *et al*., 2007) according to the following reactions:.

$$2\,CH_4 + 8\,NO_3^- \rightarrow 2\,CO_2 + 8\,NO_2^- + 4\,H_2O$$

$$3\,CH_4 + 8\,NO_2^- + 8\,H^+ \rightarrow 3\,CO_2 + 4\,N_2 + 14\,H_2O$$

Nowadays, wastewater treatment should address not only the removal of conventional pollutants but also the presence of organic micropollutants (OMPs), an issue of emerging (and increasing) concern in our societies. The effluents from wastewater treatment plants constitute the main entrance of these pollutants into the environment since conventional processes are not able to effectively remove many of these compounds (Carballa *et al*., 2004). New advanced processes have been developed in the last years to overcome the existing limitations of conventional plants. New approaches include hybrid reactors using different redox conditions, the use of carriers or adsorbents, different biomass physical conformation, etc. (Alvarino *et al*., 2016; Escolà-Casas *et al*., 2015). For instance, the combination of aerobic and anaerobic stages leads to the enhancement of the removal of several OMPs, such as venlafaxine or tramadol (Falås *et al*., 2016), whereas the use of supports enhances nitrification which has been correlated with the removal of certain OMPs, such as the anti-inflammatory ibuprofen or the hormone estradiol (Alvarino *et al*., 2014). Biomass conformation also influences OMPs removal. In the case of lipophilic OMPs, higher sorption coefficients were observed in a membrane bioreactor (MBR) compared to an activated sludge unit due to the lower biomass particle size developed in MBRs. De la Torre *et al*. (2015) compared the removal of reactors operated with suspended and attached biomasses, as well as their mixture. They observed the best results in terms of OMP removal for the IFAS-MBR (integrated fixed-film activated sludge MBR), which combine both biomasses, while the worst results were showed for the reactor operated only with attached biomass.

The SIAL system is an innovative wastewater technology with a holistic approach. This process combines different redox conditions (anaerobic, anoxic and aerobic), as well as several biomass conformations (granular, flocculent and attached) in order to minimize the methane emissions by the promotion of methanotrophic denitrification, as well as to optimize the simultaneous removal of COD, N and OMPs. This study was focused on the performance of the SIAL system, especially in terms of OMPs removal.

2. Materials and methods

The SIAL process was assessed in a pilot plant of 186 L, divided into two systems: an Upflow Anaerobic Sludge Blanket (UASB) unit of 120 L coupled to an Integrated Fixed film Activated Sludge (IFAS) polishing stage. The IFAS system contained an anoxic (36 L) and an aerobic compartment (36 and 20 L, respectively) and a final settler (10 L). The pilot plant was operated during 310 d. Both suspended and attached biomass were present in the anoxic and aerobic stages. Two different supports were used to promote the growth of attached biomass: synthetic porous foams (LEVAPOR GmbH, 20% fill ratio) in the anoxic compartment and porous semi-flexible carriers (Mutag Biochip, Multi Umwelttechnologie A.G.) in the aerobic one. In order to achieve a complete N removal, a total recirculation ratio of 3 was applied, equally divided from the settler (R=1.5) and the aerobic unit (R=1.5) to the anoxic compartment. The system was operated at a hydraulic retention time of 30 h (21.7 and 8.3 h in the UASB and in the anoxic-aerobic compartments, respectively) at ambient temperature (20-22ºC). A synthetic low strength wastewater composed by diluted skimmed milk, sodium bicarbonate and ammonium chloride was used for feeding, with an inlet COD concentration of 850 ± 100 mg·L^{-1}.

Two periods of operation were distinguished. During the first period of operation (P1), the anoxic-aerobic compartment was operated with attached and suspended biomass (days 1-167), while in the second period the reactor operated only with attached biomass, and the IFAS actually operated as a biofilm system (P2: 168-400 d).

Eleven OMPs were spiked in the influent at a concentration range of 1-20 ppb: three anti-inflammatories (ibuprofen IBP, naproxen NPX, diclofenac DCF), four antibiotics (sulfamethoxazole SMX, trimethoprim TMP, erythromycin ERY, roxithromycin ROX) and four endocrine disruptors (bisphenol BSF, estrone E1, β-estradiol E2, α-ethinylestradiol EE2). Their concentrations were followed in the influent, outlet of the UASB reactor, anoxic compartment, and final effluent. Seven sampling campaigns were carried out during fourteen months to follow up the removal of the selected OMPs during the two periods of operation.

3. Results and discussion

3.1 Reactor performance

A total COD removal was observed in the system. COD was mainly removed in the anaerobic stage (93%), with an average percentage of methanization of 75%. High removal efficiencies of dissolved methane were observed in the anoxic compartment along P1 (up to 70%), although these values decreased sharply in P2 (down to 40 %), when the anoxic-aerobic stages were operated with only attached biomass. The degradation of methane was attained mainly by the action of aerobic methanotrophs, whereas the presence of the DAMO bacteria and archaea was scarce in both periods of operation.

Partial nitritation was observed during most of P1, being the development of nitrite oxidation bacteria (NOB) not detected until the last days of this period. Nitrite acted as the main electron acceptor during P1, and only nitrate appeared at the end of this period. Moreover, during the whole P2 ammonia was fully oxidized to nitrate in the aerobic compartment. Nitrogen removal diminished from 50-60% when nitrite was accumulated (P1) to 34% in the presence of nitrate (P2). The drop on total nitrogen removal was caused by two different reasons: denitrification using nitrite has lower COD requirements, and also the denitrification rate with nitrite is higher than with nitrate. During the first days of P2, suspended biomass settling properties worsened and biomass in suspension was gradually washed out. Thus, the IFAS process was converted into a biofilm.

Denitrification in the SIAL process could be accomplished by different kind of bacteria, using different electron donors. The presence of different types of denitrifying bacteria was assessed by the FISH technique (fluorescence in situ hybridization). Denitrification observed in P1 was carried out by a heterogeneous group of bacteria comprising aerobic methanotrophs and denitrifying heterotrophs via nitrite. Moreover, these organisms, denitrifying heterotrophs via nitrate and anammox bacteria were detected in P2, principally attached on the two types of supports used.

3.2 OMP removal

The removal of the selected OMPs was studied in the SIAL system (Figure 1). SMX, TMP, and NPX were readily removed (up to 93%) under anaerobic conditions, with no significant differences in both periods. Therefore, the further effect of the aerobic and anoxic redox conditions in their removal efficiencies was not assessed due to their low concentrations after the UASB. These results are in agreement with previous studies carried out in UASB reactors used for sewage treatment (Alvarino *et al*., 2016). The high removal achieved for the natural hormones E1 and E2 was mainly related to the contribution of the anoxic-aerobic compartments. The same behavior was observed for the hormone EE2, although in this case removal efficiencies were below 70% in both periods of operation. Joss *et al*. (2004) compared the removal of the estrogens under aerobic and anaerobic conditions and observed a higher biotransformation rate with the positive redox conditions. The removal of the three hormones was stable during the whole operation, thus no effect was observed in their biotransformation related to the shift of bacterial populations in P2 (where NOB and anammox bacteria were developed). A recalcitrant behavior of IBP was observed under anaerobic and anoxic conditions, while it was readily biotransformed under aerobic conditions. Previous studies determined the influence of nitrification in IBP removal (Alvarino *et al*., 2016; Tran *et al*., 2009), very likely as a result of the availability of secondary and tertiary carbons in linear alkyl chains in its chemical structure to be hydroxylated by ammonium monooxygenase (AMO) (Fernandez-Fontaina *et al*., 2016). The slight biotransformation enhancement in P2 for IBP might be related to the action of NOB, according to Fernandez-Fontaina *et al*. (2016), who determined higher biotransformation rates of IBP when NOB activity was the predominant species in nitrification.

Although the reactor was only operated with attached biomass in P2, the removal of the antibiotics ERY and ROX was enhanced throughout this period. ERY was a recalcitrant compound in P1, while removals above 85% were achieved for this antibiotic in P2 (Figure 1). This fact might be related to the presence of anammox bacteria in P2. In fact, Alvarino *et al*., (2015) observed a direct correlation between the removal of ERY and the anammox activity. DCF was poorly removed during the whole operation of the SIAL process, with maximum removal efficiencies below 40% (Figure 1). However, a significant difference was observed among both periods. The removal of DCF achieved in P1 was related to the accumulation of nitrite in the aerobic compartment, where a correlation between nitrite concentration and DCF removal was observed (Figure 2a), thus suggesting that partial nitritation promoted the biotransformation of this recalcitrant compound. Osorio *et al*. (2016) studied the removal of DCF in a nitrification-denitrification process and detected the metabolites nitroso-DCF and 5-nitro-diclofenac in the presence of nitrite. Nitroso-DCF is produced by the nitrosation of the nitrogen atom present in the molecule of DCF, whereas the formation of 5-nitro-diclofenac is related to the transfer of a nitro group by an electron-deficient group on the aromatic ring of the phenylacetic acid (Osorio *et al*., 2014).

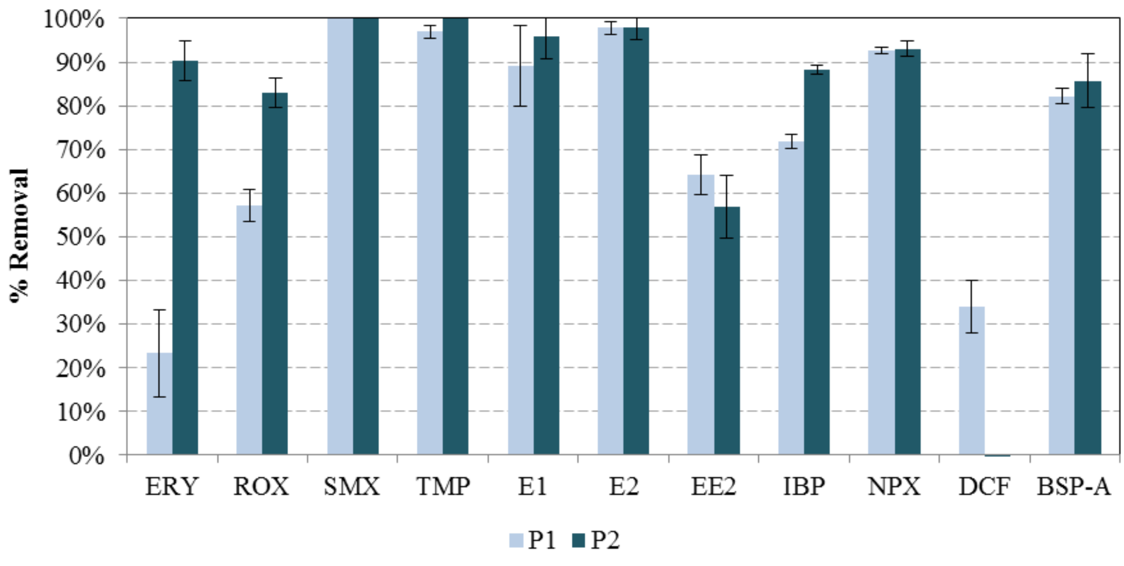

Figure 1. OMPs removal in the SIAL system in P1 and P2

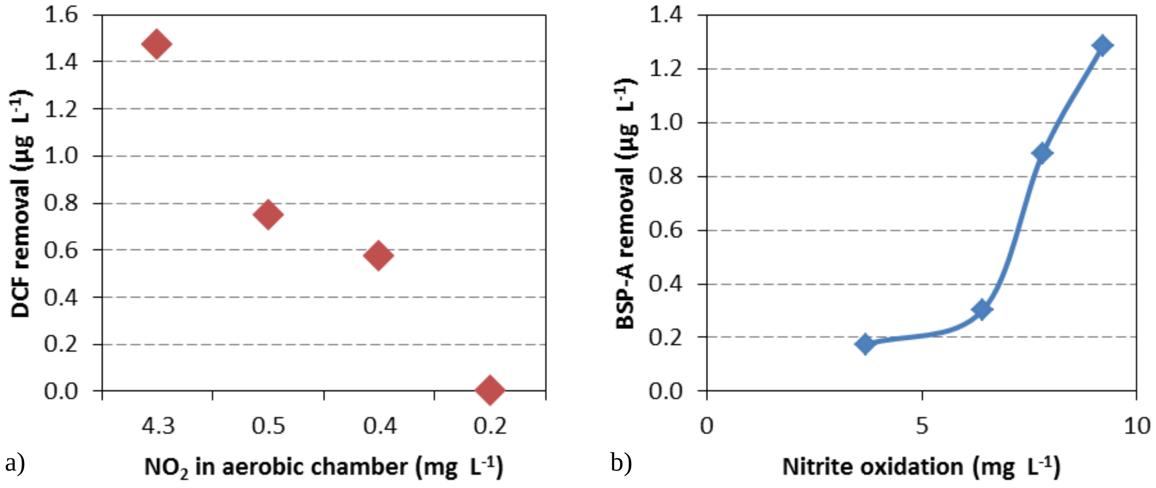

Figure 2. Influence of presence of nitrite in the aerobic compartment in the removal of DCF (a) and the nitrite oxidation in the removal of BSP-A (b)

BSP-A was readily removed in the SIAL system (Figure 1). This endocrine disruptor was recalcitrant under anaerobic conditions, thus its removal took place only in the anoxic and aerobic compartments. The BSP-A removal efficiency was positively correlated to the total nitrification (Figure 2b). This fact evidenced the effect of the nitrite oxidation bacteria in this removal. However, the overall contribution in the removal of BSP-A of the bacteria involved in the nitrification was low, thus the effect of other bacteria, such as heterotrophic bacteria, have to be considered (Zielińska *et al*., 2014). This explains the slight differences in the observed BSP-A removals during P1 and P2 (Figure 1).

4. Conclusions

The performance of a new hybrid process was assessed in terms of macro and micropollutants removal efficiencies, as well as the minimization of greenhouse gases emissions. High removals of COD and nutrients were achieved. The presence of methanotrophs in the attached and flocculent biomass present in the anoxic-aerobic compartments reduced the dissolved methane up to 70 %. The anaerobic pretreatment enhanced the removal of several OMPs, such as TMP and SMX with respect to the conventional anoxic-aerobic processes. The combination of several biomass conformations enhanced the microbial diversity, mainly under anoxic conditions. A consortium of bacteria composed by anammox, aerobic methanotrophs and heterotrophs were the main responsible species for denitrification. The growth of anammox bacteria in the attached biomass influenced positively the removal of ERY. A correlation between the presence of nitrite in the aerobic compartment and the removal of DCF was observed, while the removal of BSP-A was slightly dependent on the nitrite oxidation.

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