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Abstract: The biodegradation of ranitidine (RNT), diclofenac (DCF), and simvastatin (SVT) (50 µg/L each), in real domestic sewage, was studied. A continuous anaerobic-aerobic reactor with immobilized biomass was operated for 6 months. The hydraulic retention time (HRT) was 8 h, achieving 90, 72, and 62% removals of RNT, DCF and SVT, respectively, and 71% removal of soluble chemical oxygen demand (COD). The biodegradation products were identified by liquid chromatography coupled to mass spectrometry (LC-MS). The inhibition of the specific methanogenic activity (SMA) was evaluated by a series of batch tests. Acute and chronic ecotoxicities were estimated using the ECOSAR 1.11 software. The initial degradation rates and the maximum oxidation capacities (MOC) of the system were estimated. Even environmental concentrations of RNT, DCF, and SVT were capable of inhibiting the SMA. Lipophilicities dictated the behavior of those three drugs. The greater their lipophilicities, the greater the SMA inhibition and their ecotoxicity.

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Cover Letter

São Carlos, October 21th, 2019.

Dear *Science of the Total Environment* Editors:

Please find attached the manuscript *"Tandem anaerobic-aerobic degradation of ranitidine, diclofenac, and simvastatin in domestic sewage"*, which we are submitting to your judgment.

We state that this manuscript has not been previously published, in whole or in part, and is not under consideration by any other journal.

We believe this manuscript is worth being published as it addresses a current issue: The performance of anaerobic-aerobic treatments for degrading environmental concentrations of pharmaceuticals. In addition, this article is appropriate for publication in this journal because it is within its scope, particularly: *Waste and Water Treatment* and *Persistent Organic Pollutants*.

To our knowledge, this is the first work that assesses the performance of a combined anaerobic-aerobic bioreactor for removing ranitidine, diclofenac, and simvastatin from domestic sewage. This assessment was made sound by addressing specific methanogenic activity inhibitions, identifying biodegradation products, and estimating acute and chronic ecotoxicities. Moreover, by modeling the removal kinetics, initial rates and maximum oxidation capacities were estimated.

Thanks in advance.

Sincerely yours,

Msc. Rafaely Ximenes de Sousa Furtado
(on the authors' behalf)

*Title Page

Tandem anaerobic-aerobic degradation of ranitidine, diclofenac, and simvastatin in domestic sewage

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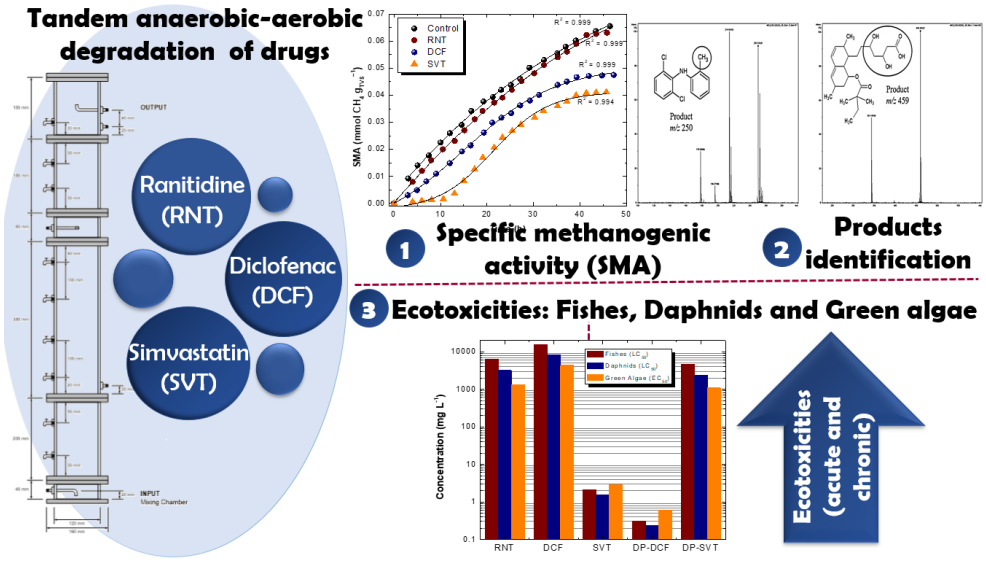
*Graphical Abstract



Highlights

- A continuous anaerobic-aerobic reactor was used in the drugs biodegradation process.
- Environmental concentrations inhibited the specific methanogenic activity.
- Acute and chronic ecotoxicities were estimated for the involved compounds.
- Initial rates and maximum oxidation capacities were estimated.
- Lipophilicity dictated the biodegradation behavior of the drugs.

Tandem anaerobic-aerobic degradation of ranitidine, diclofenac, and simvastatin in domestic sewage

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Abstract

The biodegradation of ranitidine (RNT), diclofenac (DCF), and simvastatin (SVT) (50 µg/L each), in real domestic sewage, was studied. A continuous anaerobic-aerobic reactor with immobilized biomass was operated for 6 months. The hydraulic retention time (HRT) was 8 h, achieving 90, 72, and 62% removals of RNT, DCF and SVT, respectively, and 71% removal of soluble chemical oxygen demand (COD). The biodegradation products were identified by liquid chromatography coupled to mass spectrometry (LC-MS). The inhibition of the specific methanogenic activity (SMA) was evaluated by a series of batch tests. Acute and chronic ecotoxicities were estimated using the ECOSAR 1.11 software. The initial degradation rates and the maximum oxidation capacities (MOC) of the system were estimated. Even environmental concentrations of RNT, DCF, and SVT were capable of inhibiting the SMA. Lipophilicities dictated the behavior of those three drugs. The greater their lipophilicities, the greater the SMA inhibition and their ecotoxicity.

Keywords: Ranitidine; Diclofenac; Simvastatin; Anaerobic-aerobic degradation; Specific methanogenic activity; Ecotoxicity

1. Introduction

In recent years, the impact of environmental contamination has increased mainly due to population growth, poorly planned urbanization, and the expansion of industrial and agricultural activities, which generated large volumes of wastes containing emerging organic contaminants such as pesticides, dyes, personal care products, and pharmaceuticals (Olicón-Hernández et al., 2019). The latter group is comprised of biologically active substances used for therapeutic purposes in humans and animals, which are partially excreted through urine and/or feces (Olicón-Hernández et al., 2019; Rostamizadeh et al., 2019).

The contamination of the aquatic environment by drugs occurs through numerous pathways, such as inappropriate disposal of expired drugs, discharge of hospital wastewaters or pharmaceutical industry ones, and domestic sewage (Rostamizadeh et al., 2019; Xiong et al., 2019).

The presence of drugs, such as ranitidine, diclofenac, and simvastatin (Fig. 1) in the aquatic environment has often been detected in concentrations around ng L^{-1} and µg L^{-1} (Candela et al., 2016). The presence of those substances in water startled the scientific community due to their persistence, increased concentration over time and, although some of those drugs do not directly exert toxicity, they may increase bacterial resistance or exhibit a synergistic effect through the interaction with other drugs present in the environment, representing a potential threat to the ecosystem and to humans (Mugunthan et al., 2019).

Ranitidine is widely used for treating stomach diseases including gastric and duodenal ulcers (Elias et al., 2019); diclofenac is a non-steroidal anti-inflammatory drug (NSAID) used as an analgesic, antiarthritic, and antirheumatic agent (Rostamizadeh et al., 2019); and simvastatin belongs to the statins group and reduces the levels of low-density lipoprotein

(LDL), as well as triglycerides (Mussa et al., 2016). Those drugs are resistant to biodegradation and are commonly found in surface waters (Elias et al., 2019; Rostamizadeh et al., 2019).

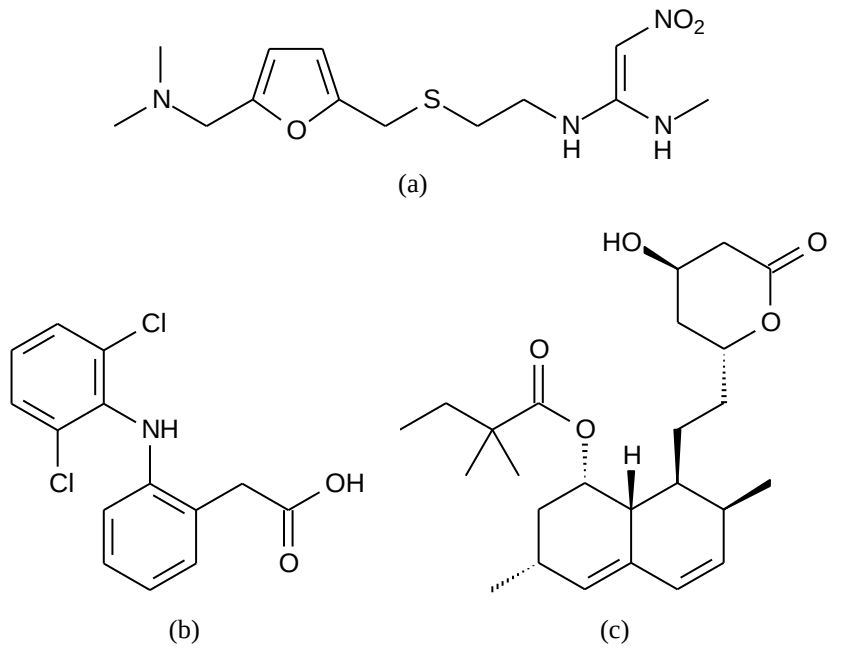

Fig. 1. Structures of the studied drugs: (a) ranitidine, RNT; (b) diclofenac, DCF; and (c) simvastatin, SVT.

Currently, decreasing drug consumption would be an unfeasible strategy in face of population growth and increased life expectancy. However, some attitudes such as: 1) investments in education, in the sense of reducing self-medication; 2) restrictions on the prescription of medicines; 3) appropriate disposal policies for expired drugs; 4) efficient wastewater treatment plants, capable of promoting the degradation of those contaminants at low concentrations; and 5) development of new biodegradable and/or less persistent drugs, may reduce environmental contamination (Kummerer, 2009).

Biological processes are being studied for the removal of synthetic compounds, such as pharmaceuticals, since many of the chemical processes capable of degrading them have high costs, especially for developing countries.

Regarding the biological treatment of domestic sewage, the activated sludge technology stands out among aerobic processes due to the generation of high-quality effluents. However, energy consumption and sludge production are high. In addition, a process exclusively based on this technology is not efficient for the removal of some emerging contaminants, among which are pharmaceuticals. For instance, in an activated sludge process followed by UV photolysis for the removal of some drugs, Salgado et al. (2012) showed that the contribution of biodegradation was 45%; photodegradation 22%, and 33% were just adsorbed onto the sludge.

On the other hand, anaerobic processes are an interesting alternative due to lower energy consumption, less sludge production, as well as the possibility of using the biogas as an alternative energy source. However, the effluents from those processes generally do not comply with discharge limits in water bodies, requiring a post-treatment step.

Therefore, the combination of anaerobic and aerobic processes has advantages over the individual processes due to the biogas production potential, high efficiency in the removal of organic matter, nutrients and solids, low sludge production, and lower energy consumption (Oliveira Netto, Zaiat, 2012). In addition to increased treatment efficiency, this configuration needs less space and can be operated near the urban area, encouraging the reuse of treated wastewater (Kassab et al., 2010).

The use of anaerobic processes with immobilized biomass has additional advantages. In the present case, cells concentration and retention time are high, contributing to process stability and increases the chances of degrading poorly biodegradable compounds, compared to the suspended biomass processes (Speece, 1983). However, those processes remain somewhat inefficient as nutrient removal only occurs in a following aerobic process. As both processes can be operated with immobilized biomass, as shown by Oliveira Netto and Zaiat

(2012), reactors can be more compact and allow for the decentralization of sewage treatment plants.

Considering this scenario, this work assesses the performance of a single biological reactor combining anaerobic and aerobic processes for removing three widely used drugs from domestic sewage. Biodegradation products were identified, the inhibition of the specific methanogenic activity (SMA) monitored, and acute and chronic ecotoxicities estimated. To our knowledge, this is the first time this kind of study is performed.

2. Materials and methods

2.1 Chemicals

Acetonitrile and methanol (Panreac, HPLC grade), ammonium acetate, sucrose, starch, cellulose, soy oil, sodium chloride, calcium chloride, magnesium chloride, sodium hydrogen carbonate, potassium hydrogen phosphate (all from Synth, > 98%), nitric acid (Merck, 65%), meat extract (Hexis), and sulfuric acid (Panreac, 96%) were used as received. Ranitidine (RNT), diclofenac (DCF), and simvastatin (SVT), were directly purchased from local distributors (purity > 99%, according to the respective certificates of analysis).

2.2 Analytical procedures

Samples were collected, cleaned by solid-phase extraction (SPE) using a polymeric reversed-phase cartridge (Strata^{TM}-X, Phenomenex), and filtered through a 0.2-µm membrane. The SPE extraction process followed the steps: 1) Conditioning – 2 mL methanol, followed by 2 mL of 0.1% formic acid (in volume) at pH 2; 2) Loading – 3 mL of the drugs solution (RNT, DCF, and SVT); 3) Washing – 2 mL of 0.1% formic acid at pH 2; and 4) Elution – 3 mL of 50% aqueous methanol solution. The method recovery values for RNT, DCF and SVT were 87.7%, 69.1% and 95.0%, respectively.

RNT, DCF, and SVT were quantified by SPE-HPLC-MS/MS. The system was comprised of a liquid chromatograph (Agilent Technologies, 1200), equipped with a quaternary pump (Infinity, 1260), autosampler (Infinity, 1260), column compartment with temperature control (Infinity, 1290), and diode array detector (DAD – Infinity, 1209). C_{18} reverse-phase chromatographic column (150 × 3 mm, 3 µm, pore size 1 Å, Phenomenex) with temperature at

40ºC and eluted with acetonitrile and 5 mmol L^{-1} ammonium acetate 10:90 in volume (gradient: 0-5 min 10-100%, 5-8 min 100%, 8-10 min 100-10%, and 10-15 min 10%), 100 μL injection volume, 0.5 mL min^{-1} flow rate, and λ = 235 nm. RNT, DCF, and SVT retention times were 4.96, 5.87, and 9.03 min, respectively.

A Q-Trap hybrid mass spectrometer (QTrap 5500, AB SCIEX) with electrospray ionization source was used to analyze the samples. Multiple reaction monitoring (MRM), using the more abundant transitions of precursors and product ions (m/z 315/102, 315/130, and 315/176 for RNT; 296/205 and 296/214 for DCF; and 436/285 and 436/419 for SVT) was employed. Source and gas parameters used in the quantification of the drugs by SPE-HPLC-MS/MS were: gas curtain, 15 psi; spray voltage, 4000 V; temperature, 700°C; heating gas, 40 psi; and nebulizer gas, 50 psi.

The detection limit (LOD) for the SPE-HPLC-MS/MS method was determined as the analytical signal three times greater than the signal-to-noise ratio; the quantification limit (LOQ) was the first point of the analytical curve, with an analytical signal ten times greater than the signal-to-noise ratio. Therefore, the estimated LOD and LOQ were 10, 15, and 50 ng L^{-1} and 50, 50, and 200 ng L^{-1} for RNT, DFC, and SVT, respectively.

Chemical oxygen demand (COD) was determined by closed reflux (method 5220D) and solid analyses were performed by gravimetry (method 2540), according to the procedures described in the Standard Methods for the Examination of Water and Wastewater (APHA; AWWA; WEF, 2005). Dissolved oxygen (DO) in the aerobic chamber of the biological reactor was quantified using a portable oximeter (MO-900, Instruterm®).

The concentrations of nitrite, nitrate, ammonium, and phosphate were determined by ion chromatography, using a chromatograph (ICS 5000, Dionex) equipped with conductivity

detector and two columns (IonPac AG23 Anion-Exchange and IonPac CG12A Cation Exchange), operating at 30ºC and 1 mL min^{-1} flow rate. Mobile phases were a mixture of 4.5 mmol Na_2CO_3 L^{-1} and 0.8 mmol $NaHCO_3$ L^{-1} (anions) and 40 mmol H_2SO_4 L^{-1} (cations).

The amount of methane formed during the biodegradability tests was determined by a gas chromatograph (GC2014, Shimadzu) equipped with a HP-PLOT Q column. Volatile acids were determined according to Dilallo and Albertson (1961) and the alkalinity according to Ripley, Boyle, and Converse (1986).

2.3 Batch anaerobic reactors

2.3.1 Identification of biodegradation products

The biomass used in the batch anaerobic tests was obtained from an upflow anaerobic sludge blanket (UASB) reactor operated at Monjolinho's wastewater treatment station (WWTS), which treats the domestic sewage of São Carlos city, São Paulo, Brazil.

First, the sludge was collected and acclimatized to a simulated sewage (mg L^{-1}): sucrose, 47.8; starch, 149; cellulose, 47.2; meat extract, 215; soy oil, 51; $NaHCO_3$, 728; K_2HPO_4, 15; NaCl, 250; $MgCl_2.6H_2O$, 7; and $CaCl_2$, 4.5 — prepared as described by Gomes et al. (2015). The resulting COD was approximately 500 mg O_2 L^{-1}.

Second, nitrogen gas was bubbled for 5 min in the borosilicate bottle reactor containing the washed sludge and the simulated sewage (10% washed sludge, 70% simulated sewage, and 20% headspace, all percentages in volume) to eliminate the dissolved oxygen. Afterwards, the reactor was closed with a butyl septum and kept under stirring (150 ± 2 rpm)

at 30ºC. The sludge was considered to be acclimatized when the COD removal was constant and the drugs contained in the bulk were no longer detected.

Third, the sludge was split into 12 borosilicate bottles: 3 with the individual drugs (RNT, DCF, or SVT) and 1 with the control (sludge and simulated domestic sewage), in triplicate. Those bottles were initially operated during 30 days under the same conditions as the sludge acclimation assay.

In order to favor the detection of the biodegradation products, high concentrations of the drugs were used (few mg L^{-1}) — they were increased each week: 0.05, 1.5, 3.0, and 5.0 mg L^{-1}. After that, the drugs were added only at 5.0 mg L^{-1}, until their removal stabilized (monitored by SPE/HPLC-MS/MS). The most intense peaks of the drugs biodegradation products were analyzed by MS/MS and the structures were suggested with the aid of the Data Analysis software (Bruker).

2.3.2 Biodegradability tests

As the drugs affected the anaerobic degradation process (different COD removals during the batch experiments), biodegradability tests were performed and expressed as SMA, in mmol CH_4 g_{TVS}^{-1}, where TVS means total volatile solids. Then, a sigmoidal (Boltzmann) model was adjusted to the experimental data and Equation 1 was used to calculate the time-averaged SMA, in mmol CH_4 g_{TVS}^{-1} h^{-1}. Those tests were important for determining the methanogenesis inhibition due to the added drugs.

$$Time\text{-}averaged\ SMA = \frac{\int_0^t SMA\ dt}{\int_0^t dt} \quad (1)$$

227
228 The procedure was similar to that used to identify the degradation products, except for
229 the drugs concentration (50 μg L^{-1}) and the run time (48 h). Samples were withdrawn from
230 the headspace at given times and the amount of generated CH_4 was determined by gas
231 chromatography.

232 *2.4 Ecotoxicity estimates*

233 Acute and chronic ecotoxicities towards three trophic levels (fishes, daphnids, and green
234 algae) were estimated (*in silico*) using the ECOSAR 1.11 software (USEPA, 2017). Acute
235 ecotoxicities indicate the LC_{50} (lethal concentration to 50% of the tested organisms) and the
236 EC_{50} (effect concentration to 50% of the tested organisms). Chronic ecotoxicities were
237 estimated as the geometric mean of the LOEC (lowest-observed-effect concentration) and the
238 NOEC (no-observed-effect concentration).

239 *2.5 Anaerobic-aerobic continuous flow reactor fed with a domestic sewage*

240 The anaerobic-aerobic reactor (fixed bed, upward flow) shown in Fig. 2 was operated
241 during 6 months at *Campus* 2 of the University of São Paulo (USP), São Carlos city, Brazil,
242 where the domestic sewage of the neighborhood can be collected. Coarse solids were
243 removed by a screen system and then the sewage was pumped into a 1000 L reservoir that fed
244 the reactors.

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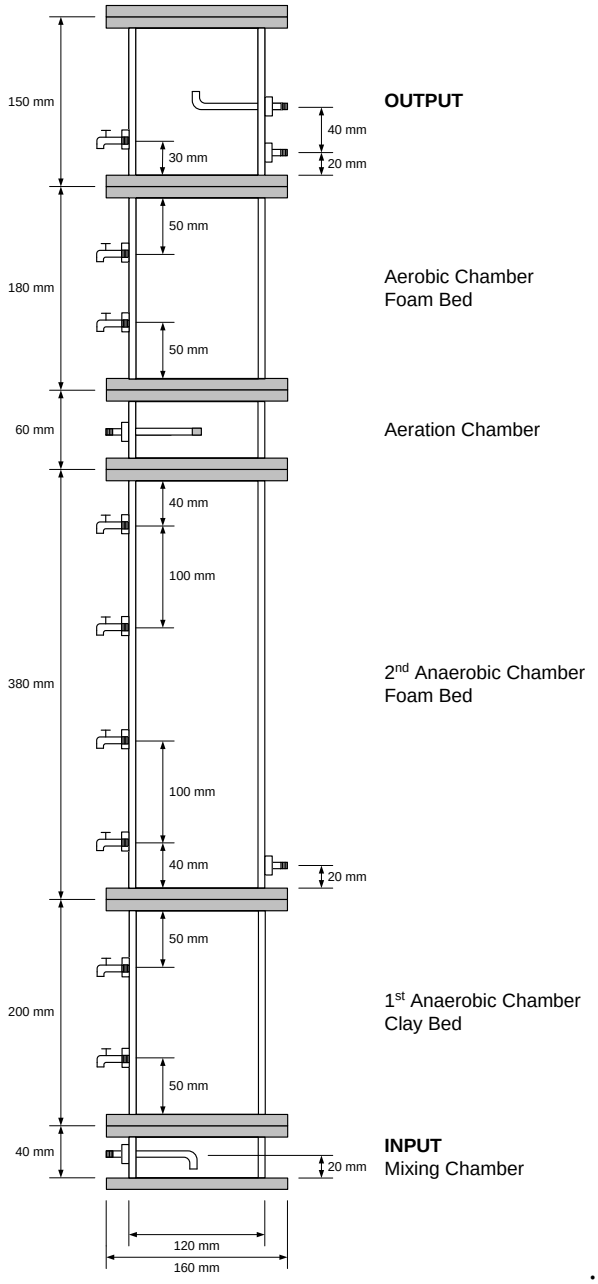


Fig. 2. Details of the reactor (acrylic) used in the biological treatment of the domestic sewage.

The reactor bed was comprised of two support materials: i) expanded clay (average particle size 10 ± 5 mm) in the first anaerobic chamber and ii) polyurethane foam cubes (apparent density 23 kg m^{-3}, pore size 543 ± 154 µm, specific surface area 43.8 m^2 g^{-1}, and edge 1 cm) in the second one. Expanded clay was used in the first chamber because it favors the adhesion of acidogenic microorganisms that produce the substrates for the microbial community adhered to the polyurethane foam (Ribeiro et al., 2003).

The first anaerobic chamber (expanded clay) was not previously inoculated; the second one was inoculated with biomass from an UASB reactor in operation — Piçarrão WWTS, treating the domestic sewage of Campinas city, São Paulo, Brazil. This procedure consisted in adding the sludge, in suspension, and the support material (polyurethane foam) to a 5-L recipient and leaving them in contact for 2 h (room temperature = 30ºC). Poorly adhered biomass was removed from the foam cubes by washing them with the domestic sewage itself (Zaiat et al., 1996). Afterwards, the inoculated foam was placed in the second anaerobic chamber of the reactor, which was continuously fed with domestic sewage. Hydraulic retention times (HRT) were 18 h in the first week, decreasing to 16, 14, and 12 h in the following three weeks, respectively. Later on, the HRT was further decreased to 8 h. Then, a spatial COD profile was obtained when this parameter stabilized in the reactor effluent. This reactor was first designed and operated by Oliveira Netto and Zaiat (2012).

The removal kinetics of RNT, DCF, and SVT were determined based on the model proposed by Chan and Chu (2003), shown in Equation 2, in which C is the concentration of the drug at time t (h), C_0 is the initial concentration of the drug, ρ (h) and σ (dimensionless) are two characteristic constants related to the reaction kinetics and the oxidation capacity, respectively.

$$\frac{C}{C_0} = 1 - \frac{t}{\rho + \sigma t} \quad (2)$$

The slope of Equation 2 at any time can be mathematically determined by taking its derivative in time (Equation 3).

$$\frac{d(C/C_0)}{dt} = -\frac{\rho}{(\rho + \sigma t)^2} \quad (3)$$

When t is zero, the slope is then $-1/\rho$ (h^{-1}) and its physical meaning is the initial (pseudo-first-order) removal rate of the drug. Here, the removal rate has no concentration unit because C/C_0 is dimensionless.

On the other hand, when t is long enough, approaching infinity, $1/\sigma$ is the theoretical maximum removal fraction, which is equivalent to the maximum oxidation capacity (MOC) of the system (Equation 4).

$$\frac{1}{\sigma} = 1 - \frac{C_{t\to\infty}}{C_0} \tag{4}$$

The characteristic constants (ρ and σ) were estimated by the Solver package (Excel® software) using the Generalized Reduced Gradient (GRG) nonlinear solving and least squares methods.

3. Results and discussion

3.1 Batch anaerobic reactors

3.1.1 Identification of biodegradation products

The samples were analyzed at different treatment times in order to assess the formation of different degradation products. However, only one biodegradation product of DCF and one of SVT (Fig. 3) were identified. The lipophilicities (log *D*) of the drugs and their degradation products were estimated by the Chemicalize online platform (CHEMICALIZE, 2019).

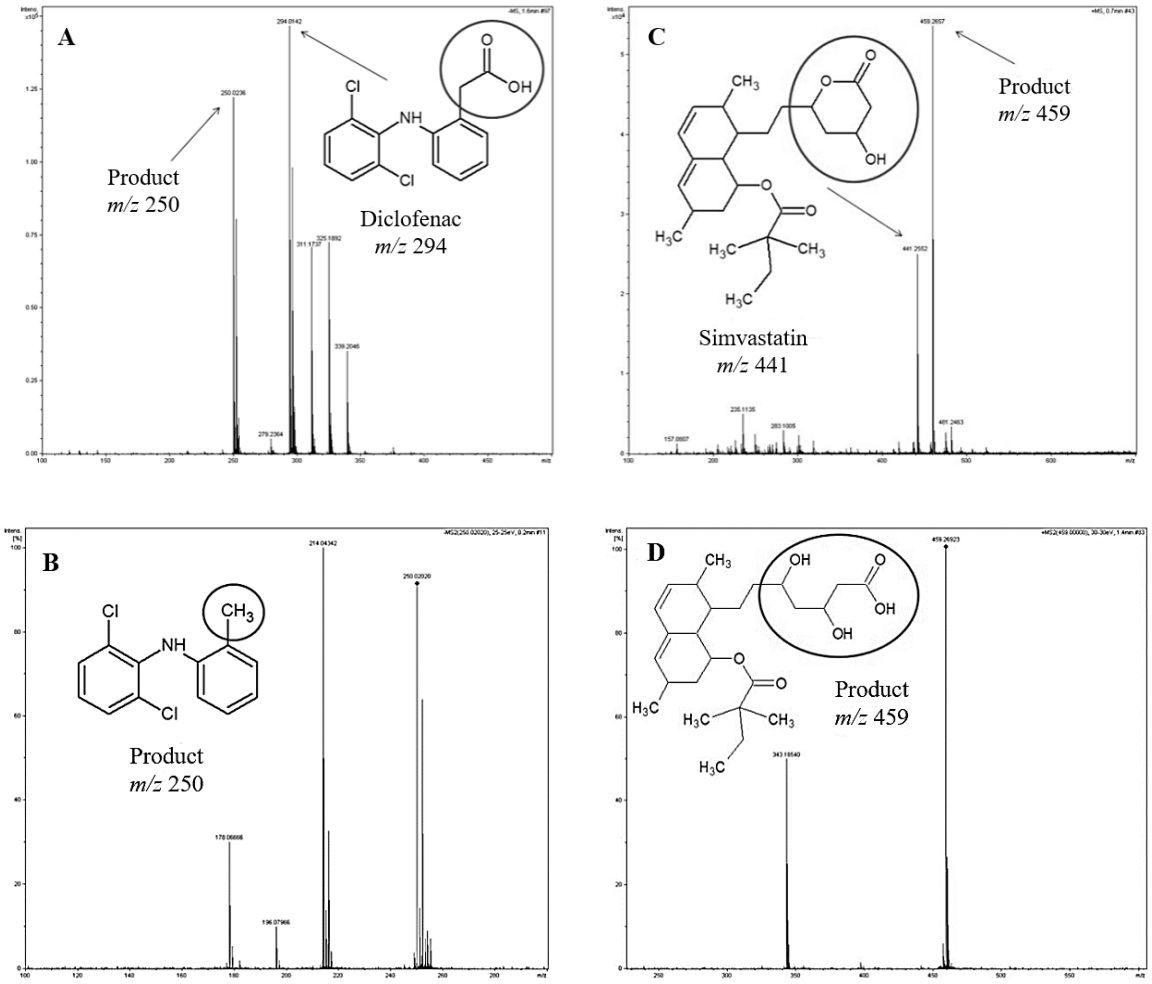


Fig. 3. Identification of products formed in the biodegradation of DCF ($[M-H]^-$) and SVT ($[M+Na]^+$): (a) Full scan negative mode of sample containing only DCF and products; (b) MS/MS product m/z 250; (c) full scan negative mode of sample containing only SVT and products; and (d) MS/MS product m/z 459.

Perhaps no RNT (log $D_{pH=7.6}$ 0.58) biodegradation products were detected because they were mineralized, unlike the ones formed from DCF and SVT, which were not biodegraded during this process.

During the anaerobic biodegradation process of DCF (log $D_{pH=7.6}$ 1.00), a decarboxylated degradation product was observed (log $D_{pH=7.6}$ 5.14). Considering the huge lipophilicity increase (approximately four orders of magnitude) and that dechlorination products were not found, one may suggest that the effluent ecotoxicity might have been increased due to the formation of more hydrophobic compounds.

On the contrary, regarding SVT (log $D_{pH=7.6}$ 4.46) one hydrolysis degradation product was identified: simvastatin acid (log $D_{pH=7.6}$ 0.59), major metabolite of SVT (Xu et al., 2014; Munaga et al., 2016). According to Alvarez-Lueje (2005), the hydrolysis of lactones is favored at pH close to 7. Thus, the identified hydroxyacid might have been formed with the aid of hydrolytic enzymes in the initial stages of the anaerobic biodegradation process. This time, a huge lipophilicity decrease took place (again, approximately four orders of magnitude), possibly indicating an ecotoxicity reduction.

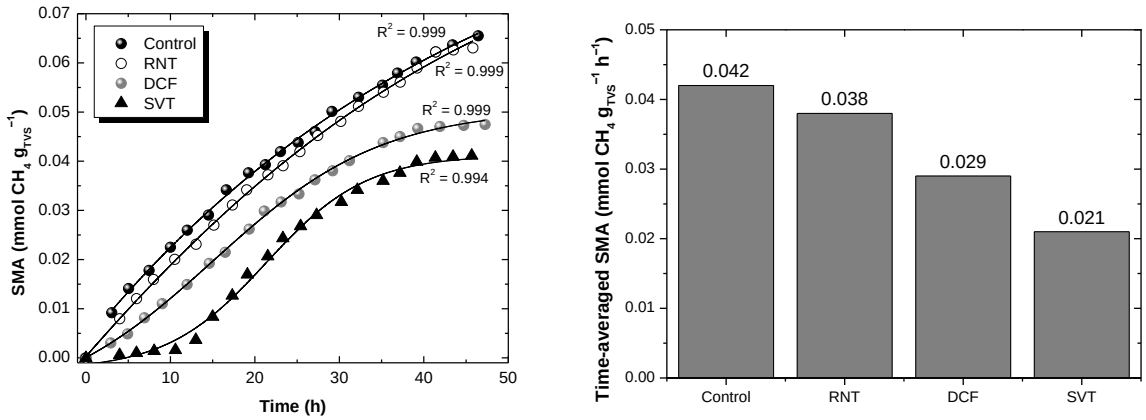
Those findings highlight the complex nature of the anaerobic degradation process and the resultant importance of identifying degradation products in the effort of gathering as much information as possible for the elucidation of biotransformation routes.

3.1.2 Biodegradability tests

To better understand the degradation process, one determined the amount of drugs adsorbed on the sludge. For that purpose, the sludge was inactivated, as described by Vela et al. (1999) .

Initially, the TVS concentration in the reactor was determined according to Ribeiro et al. (2005): 3.66 g L^{-1}. The batch reactors containing the inactivated sludge, simulated sewage, and the three drugs (50 μg L^{-1}) were kept in a temperature-controlled shaker (150 ± 2 rpm, 30 ± 1ºC) for 12 h. The drugs concentration was determined in the liquid phase and 8% RNT, 44% DCF, and 53% SVT were removed by adsorption, showing that RNT is mainly removed by biodegradation, unlike DCF and SVT. That is in accordance with their respective lipophilicities.

Prior to monitoring the SMA, blank experiments were carried out without any carbon source to quantify methane production by endogeny, which was subtracted from the results of the biodegradability tests. The results are shown in Fig. 4.



(a) (b)

Fig. 4. Specific methane activity: (a) SMA of the biomass (solid lines represent the adjusted Boltzmann model) and (b) Time-averaged SMA inhibition.

It took more time for the beginning of methane production with the addition of SVT than with DCF or RNT. That maybe because simvastatin acid is more polar than SVT. Therefore, the former has less interaction with the biomass.

The SMA was affected by the drugs even at low concentrations (50 μg L^{-1}), especially with DCF and SVT. This is probably related to the more lipophilic character of both drugs.

Regarding RNT, the ready reduction of the nitro group is known to occur in anaerobic environments (Crocker et al., 2006; Kornberger et al., 2009). That would be responsible for a dramatic detoxification towards methanogens. That is probably because the SMA of the control is quite similar to the one obtained in the presence of RNT.

In fact, the ability of anaerobic consortia to remove and detoxify the nitro group would make anaerobic processes a useful treatment adjunct and/or alternative to conventional aerobic systems. Dolon et al. (1995) argue that the anaerobic reduction of nitro groups may be an important initial step, when followed by an aerobic post-treatment. That combination could result in the complete mineralization of highly nitrated compounds as trinitrotoluene and picric acid, which are highly resistant to aerobic degradation.

The lipophilicity of a compound is directly related to its partitioning into bacterial membranes. Therefore, one should expect higher toxicities from lipophilic substances. As they accumulate in membranes, swelling, leaking, disrupted ion gradients, and eventually cell lysis may occur. On the other hand, some functional groups may react with proteins, inhibiting enzymes (Donlon et al., 1995).

That inhibition may also depend on the specific transport system of the microorganism (Olguin-Lora et al., 2003). Moreover, the SMA may be impaired, as methanogens rely almost entirely on membrane potential (H^+ and Na^+ gradients) to obtain energy during their metabolism (McMillan et al., 2011).

Therefore, lipophilicity and the intrinsic reactivity of a certain compound, besides the transport system of the cell, should be taken into consideration when assessing its toxic potential or SMA inhibition, as far as anaerobic consortia are concerned.

In order to achieve good correlations between lipophilicities and any effect upon cells, one should use the right partition coefficient. P (or K_{OW}) measures the partitioning of a compound between two immiscible solvents. In practice, the system octan-1-ol/water is used. That partition coefficient describes the lipophilicity of neutral compounds, or of the ones that exist in a single form.

On the contrary, ionizable compounds may exist in a variety of species, depending on the surrounding pH. In such cases, D (the distribution coefficient) is the correct one. As both coefficients may span several orders of magnitude, they are typically used in the logarithmic form: log P (or log K_{OW}) and log D.

3.2 Ecotoxicity estimates

Acute and chronic ecotoxicity estimates were made taking into consideration the impact treated domestic sewage (containing drugs) might have upon water bodies. Therefore, acute and chronic ecotoxicities of the studied drugs and their identified degradation products towards three trophic levels (fishes, daphnids, and green algae) were estimated from their chemical structures using the ECOSAR 1.11 software (Fig. 5).

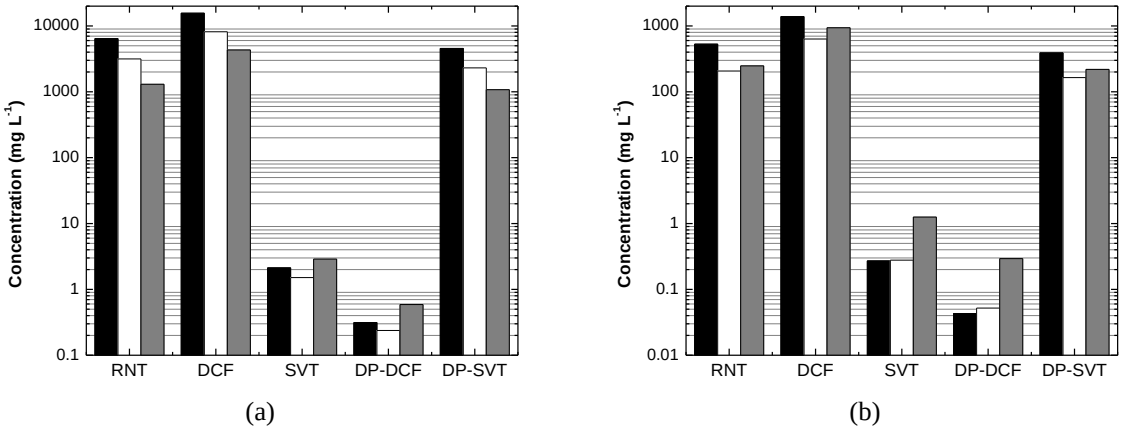


Fig. 5. a) Acute and (b) chronic ecotoxicities estimation using ECOSAR 1.11: ■ Fishes (LC_{50}), □ Daphnids (LC_{50}), and ■ Green Algae (EC_{50}).

Similar ecotoxicity profiles were achieved, whether acute or chronic ones. The main difference is that concentrations exerting chronic effects were, in average, one order of magnitude lower.

It seems that, in the case of those three drugs, lipophilicities were more important than structural differences in determining their ecotoxicity. RNT and DCF have similar ecotoxicities and lipophilicities. SVT was both three orders of magnitude more ecotoxic and more lipophilic.

Regarding the identified biodegradation products, the same pattern shows up. DP-DCF was four orders of magnitude more ecotoxic and also more lipophilic. On the other hand, DP-SVT (simvastatin acid) was three orders of magnitude less ecotoxic and less lipophilic.

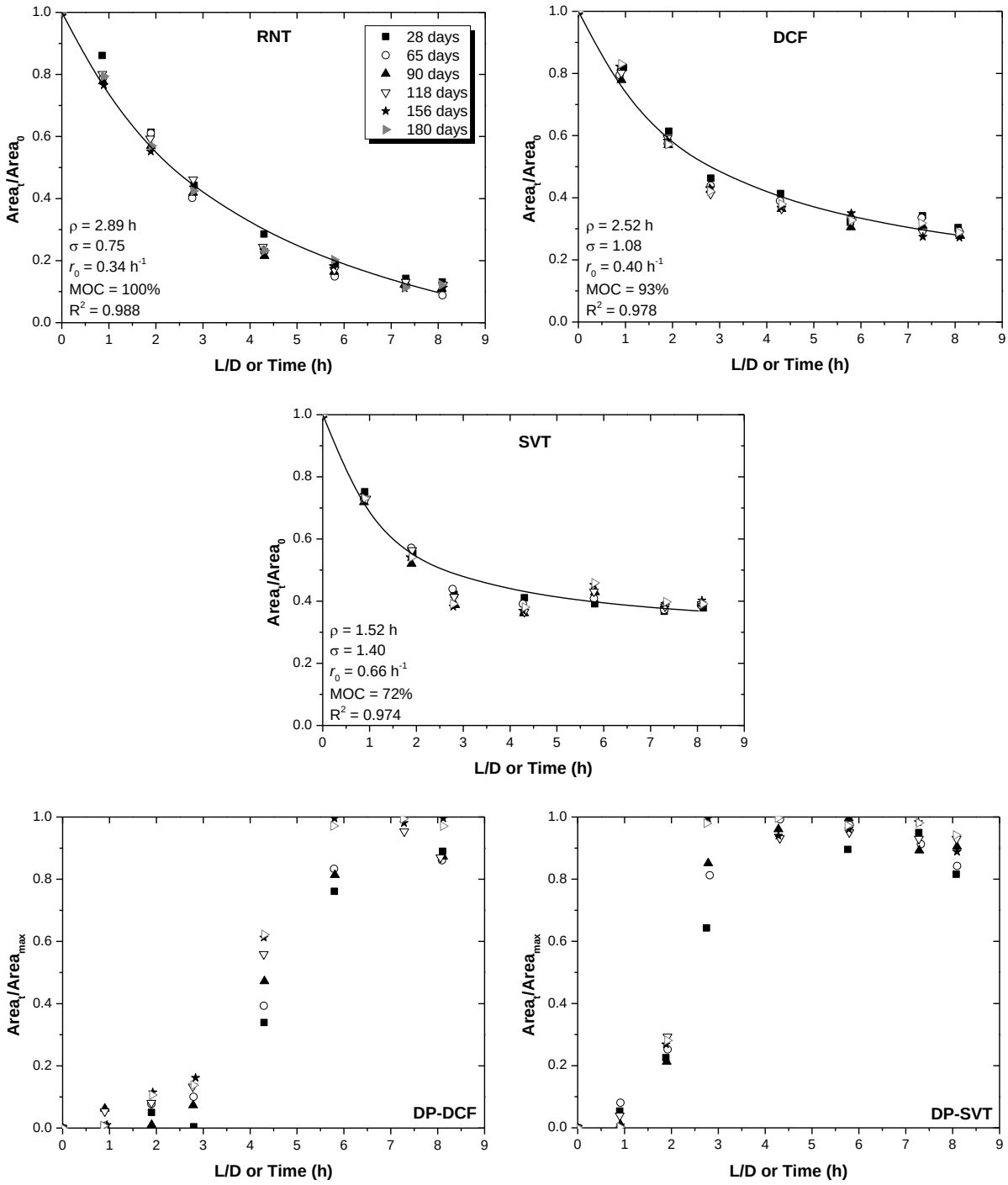
The effect of structural differences may be observed when one compares RNT and DCF. Although DCF is approximately two times more lipophilic than RNT, the former is less ecotoxic than the latter.

3.3 Anaerobic-aerobic reactor and drugs degradation

The anaerobic chambers were gradually operated increasing the organic load, which was achieved by decreasing the respective HRT (18, 16, 14, and 12 h). This strategy acclimatized the methanogenic community to the volatile acids concentration before being submitted to more critical conditions. HRT changes occurred whenever COD removals became stable. COD removals were approximately 80 and 70%, for total and soluble COD, respectively.

425 During that period, pH, alkalinity, and volatile acids concentration of the effluent were
426 also monitored (approximately 7.6, 175 mg $CaCO_3$ L^{-1}, and 30 mg CH_3COOH L^{-1},
427 respectively), as these parameters could have shown any imbalance of the process.
428
429 The strategy of gradually increasing the organic load made the biomass adaptation
430 easier. This can be observed by the high alkalinity of the effluent, showing the conversion of
431 the organics into CO_2. Under those conditions, the methanogenic community was able to use
432 the volatile acids, leaving the effluent pH slightly higher than the affluent one.
433
434 The HRT was further decreased, aiming at increasing the reactor treatment capacity.
435 Tested HRTs were 10, 8, and 6 h. When the HRT was 6 h, soluble COD removal dropped to
436 59%, while at 10 and 8 h it remained at 70%. Therefore, the HRT was fixed at 8 h and the
437 operation of the aerobic chamber was started.
438
439 After two weeks, the combined remained stable. The process efficiency in terms of
440 soluble COD removal was 93% (remaining COD = 32 ± 6 mg L^{-1}). The oxidation of nitrogen
441 compounds was also observed: $N\text{-}NO_3^-$ and $N\text{-}NO_2^-$ concentrations in the effluent of the
442 anaerobic chamber were 10.7 and 3.3 mg L^{-1}, respectively. After the aerobic step, the
443 concentrations of those anions were 63.0 and 12.1 mg L^{-1}, respectively. Phosphate
444 concentration after the anaerobic step was 19 mg L^{-1}. However, it was completely removed
445 after the aerobic step.
446
447 Once the reactor was stable, the drugs were continuously added to the sewage, so that
448 their concentration in the mixing chamber was 50 μg L^{-1} each. That concentration was
449 chosen so that the target compounds (RNT = 88 ng L^{-1} and DCF= 126 ng L^{-1}) already present
450 in the sewage had no significant effect on the initial concentration of the influent of the

451 reactor. It took approximately 20 days for the degradation to become stable, with 90, 72, and
452 62% of RNT, SVT, and DCF removals, respectively. The concentration profiles of the drugs
453 (and their degradation products) were monitored along the reactor during 6 months (Fig. 6).
454



455 **Fig. 6.** Spatial (or temporal) profiles of the drugs and biodegradation products in the anaerobic-aerobic reactor
456 during 180 days of operation. Solid lines: adjusted Chan and Chu (2003) model. Modeling parameters: ρ and σ.
457 Modeling results: r_0, MOC, and R^2. L/D: length/diameter ratio. $Area_t/Area_0$: ratio between measured (in time *t*)
458 and the initial chromatographic peak areas of each compound. $Area_{max}$: the greatest chromatographic peak area
459 obtained for each compound.
460

First, one must bear in mind the following correlation between L/D ratios (Fig. 6) and reactor sections (Fig. 2): 0 – Input (mixing chamber); 1 and 2 – 1st Anaerobic Chamber (clay bed); 3, 4, and 5 – 2nd Anaerobic Chamber (foam bed); 6 and 7 – Aerobic Chamber (foam bed); and 8 – Output.

Second, it is important to notice that "degradation" just means that the target molecule underwent some kind of (bio)chemical transformation, so that it is no longer detected by the analytical method employed. Third, drugs or DPs concentrations were measured in the liquid phase. That means non-adsorbed or desorbed molecules.

Among RNT, DCF, and SVT, RNT was the easiest one to be degraded, throughout the whole reactor (anaerobic + aerobic sections). In fact, one could adjust a single first-order model to the experimental data ($k = 0.29\ h^{-1}$, $R^2 = 0.977$), confirming that its degradation pattern was approximately the same along the reactor. Moreover, RNT degradation was the least affected by adsorption-desorption phenomena, as only 8% was adsorbed on the inactivated sludge.

The performed modeling showed that RNT had the smallest initial degradation rate among the studied drugs. That is probably because significant amounts of DCF and SVT are initially removed by adsorption, making the correspondent "degradation" rates artificially high.

The estimated MOC showed that the proposed reactor would have been capable of completely removing RNT, if it was long enough or the HRT increased. Indeed, 90% removal was achieved at the output of the reactor.

Although DCF is mostly considered persistent under anaerobic conditions (Falås et al., 2016), it was degraded (decarboxylated) in the anaerobic chambers of the reactor in a considerable extent (72%). That is a somewhat surprising result, as DCF decarboxylation, which can theoretically occur under anaerobic conditions, has been observed during aerobic ones. Moreover, no reductive dechlorination reactions were observed (Jewell et al., 2016).

However, it may be misleading to compare results obtained with very low contaminant concentrations, regarding other available carbon sources. In that case, cometabolic processes may play a crucial role, being responsible for non-expected degradations of the contaminant (Ternes, Joss, 2008).

Besides that, the huge increase in lipophilicity and toxicity that occurred when DCF was turned into DP-DCF, probably impaired the continuity of its degradation in the aerobic chamber.

The estimated MOC for DCF was quite high (93%), although the achieved removal was only 72%. Maybe the deleterious effect of DP-DCF upon the aerobic consortium was responsible for that.

Silva et al. (2006) and Oliveira-Netto and Zaiat (2012) showed that the use of clay (1st anaerobic chamber) favors hydrolysis and acidogenesis, while polyurethane foam (2nd anaerobic chamber) mainly favors acetogenesis and methanogenesis. That explains why SVT was only degraded in the first chamber of the reactor, as the identified degradation product of SVT was simvastatin acid, generated by the hydrolysis of the lactone moiety of the molecule.

512 Even though simvastatin acid is much more hydrophilic than SVT, probably the high
513 lipophilicity of the latter prevented it from being extensively degraded. The estimated MOC
514 was only 72%. In the reactor effluent, one achieved 62% SVT removal.

4. Conclusion

- RNT degradation products were readily mineralized, whereas the ones from DCF (2,6-dichloro-*N*-(2-methylphenyl)aniline) and SVT (simvastatinic acid) were recalcitrant.

- Those three drugs lipophilicities, rather than their structural differences, dictated their behavior. The greater the lipophilicity the greater the SMA inhibition and their ecotoxicity.

- The proposed combined reactor was potentially capable of completely removing RNT, but not DCF and SVT.

- Each drug was degraded in different sections of the reactor: RNT – along the three chambers (anaerobic + aerobic ones); DCF – mainly in the two anaerobic chambers; and SVT – only in the first anaerobic chamber (hydrolysis step).

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***conflict of Interest Statement**

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: