Brazilian Journal of Chemical Engineering (2022) 39:35–46
https://doi.org/10.1007/s43153-021-00178-3

ORIGINAL PAPER

Can different inoculum sources influence the biodegradation of sulfamethoxazole antibiotic during anaerobic digestion?

Caroline F. Sella[1] · Rodrigo B. Carneiro[2] · Carolina A. Sabatini[1] · Isabel K. Sakamoto[1] · Marcelo Zaiat[1]

Received: 4 May 2021 / Revised: 19 October 2021 / Accepted: 20 October 2021 / Published online: 5 November 2021

Abstract

Sulfamethoxazole (SMX) is one of the antibiotics most frequently detected in effluents from conventional wastewater treatment plants, which increases the concern about the possible impacts on the aquatic biota and public health regarding the emergence of bacteria resistant to this drug. Anaerobic fixed bed reactors are supposed to enhance antibiotic biodegradation due to the biofilm formation in the reactor. In this context, this study evaluated the dynamics of the microbial community in the biofilm of three inoculum sources taken from Upflow Anaerobic Sludge Blanket (UASB) reactors for the biodegradation of SMX in anaerobic structured bed biofilm reactor (ASBBR) with: poultry slaughterhouse sludge (PS), brewery sludge (BS) and domestic sewage sludge (SS). The ASBBR reached high COD (Chemical Oxygen Demand) removal (> 84%) and biomethane yield (> 276 mLCH_4 g^{-1} $COD_{removed}$) for all inocula. The bioreactor operation with PS inoculum presented the best SMX removal (90 ± 5%), while the BS and SS inocula resulted in 84 ± 6% and 70 ± 5% removal, respectively. The kinetic profiles of COD and SMX removal indicated the occurrence of cometabolic biodegradation of sulfonamide. The molecular biology analysis showed that the microbial community of the SS inoculum suffered significant changes during the ASBBR operation for the Archaea and Bacteria domains and the biomass of PS presented more similarity to the inoculum, indicating a better SMX adaptation, in agreement with the higher SMX removal. Furthermore, the diversity of the Archaea domain (mainly *Methanosaeta* and *Methanosarcina*) increased in the biomass after each reactor operation compared to the raw inocula, indicating that the methanogenic pathway was favored during the anaerobic digestion. The experimental results showed that the inoculum source plays an important role in the SMX biodegradation during the biological wastewater treatment.

Keywords Anaerobic structured bed biofilm reactor · Brewery sludge · Kinetics · Poultry slaughterhouse sludge · Sewage sludge · Sulfonamides

✉ Rodrigo B. Carneiro
rodrigocarneiro@sc.usp.br

Caroline F. Sella
caroline.sella@usp.br

Carolina A. Sabatini
carolinasabatini@gmail.com

Isabel K. Sakamoto
sakamoto@sc.usp.br

Marcelo Zaiat
zaiat@sc.usp.br

[1] Biological Processes Laboratory (LPB), São Carlos School of Engineering (EESC), University of São Paulo (USP), 1100, João Dagnone Ave., Santa Angelina, São Carlos,, São Paulo 13563-120, Brazil

[2] Laboratory of Chromatography (CROMA), Institute of Chemistry of São Carlos, University of São Paulo (USP), 400, Trabalhador São-Carlense Ave., São Carlos, São Paulo 13566-590, Brazil

Introduction

The discovery of antibiotics brought many advances in human and veterinary medicine to combat morbidity and mortality from infectious diseases. In the past few decades, however, studies such as the one performed by van Boeckel et al. (2014) showed a growth in antibiotics consumption caused by self-medication, overdose, prophylactic, among other factors. Due to this, the presence of these pharmaceutical compounds is increasingly being detected in domestic sewage, effluents from wastewater treatment plants (WWTPs), surface waters (range from ng L^{-1} to µg L^{-1}), soils (ng g^{-1}) and sludge from WWTPs (range from ng g^{-1} to µg g^{-1}) (Danner et al. 2019; Gworek et al. 2021; Langbehn et al. 2021; Park et al. 2020; Singh et al. 2019; Su et al. 2021). Antimicrobial compounds released into the environment occur from the production stages to after their

use, since part of these pharmaceuticals is eliminated via animal and human excreta or discarded directly into sewage collection systems (Reis et al. 2020). The exponential growth in the use of antibiotics and their availability in the environment are among the main causes of the development of bacterial resistance, which means an immense global public health danger as more infections cannot be treated by known antibiotics (Grenni et al. 2018; Singh et al. 2019).

Sulfamethoxazole (SMX) is a sulfonamide antibiotic bacteriostatic that competitively inhibits dihydropteroate synthase, preventing the formation of dihydropteroic acid, an intermediate of tetrahydrofolic acid (THF) synthesis. It is mainly used in association with trimethoprim, inhibiting bacterial cell growth by preventing the synthesis of tetrahydrolic acid and is used to treat respiratory, gastrointestinal, renal and urinary tract infections (Yun et al. 2012). SMX is frequently detected in sanitary sewage worldwide (main range from 80 to 450 ng L^{-1}) and in WWTP effluents (main range from 25 to 250 ng L^{-1}) (Carneiro et al. 2019).

Alternatively, some processes are potential treatment methods for enhancing the removal of antibiotics in conventional WWTPs, e.g., advanced oxidation, ozonation, ultraviolet radiation, membrane filtration and activated carbon fiber (Cerreta et al. 2020; Du et al. 2020; Liu et al. 2020; Shahmahdi et al. 2020). The implementation and operation costs for these systems are quite high, mobilizing efforts so that new and more economical treatment possibilities are investigated. Among these, biological processes with acclimated activated sludge and aerobic membrane bioreactors have achieved good results in removing these antimicrobial compounds (Wang and Wang 2018; Zhao et al. 2018).

Anaerobic bioreactors are favored in warm climatic conditions and have been used successfully as the main unit in several WWTPs in Brazil and other Latin American countries, reducing the organic load in domestic and industrial wastewater, and generating biogas, which can be used as an energy source (Chernicharo et al. 2015). Anaerobic fixed-bed biofilm reactors (AFBBR) have been studied as an alternative to the conventional systems, e.g., Upflow Anaerobic Sludge Blanket (UASB) reactors and anaerobic stabilization ponds, and consist of bioreactors with inert support material for biofilm attachment and growth. This configuration has the advantage of avoiding the washout of biomass maintaining a high sludge retention time (from 55 to 230 d) (Carneiro et al. 2020), thus increasing the resistance to toxic compounds, as well as the biodegradation efficiency (Monteoliva-García et al. 2020). AFBBR has proved to be a promising technology to biodegrade SMX. Carneiro et al. (2019) observed 83% of SMX removal from domestic sewage under a hydraulic retention time (HRT) of 12 h in an anaerobic structured bed biofilm reactor (ASBBR) and Chatila et al. (2016) achieved 97% of SMX removal in a horizontal-flow anaerobic immobilized biomass (HAIB) reactor operated with a HRT of 24 h. The variation in the efficiency of the studies points to the need to elucidate the factors which might improve the performance of the bioreactors. These differences in performance can be strictly related to the reactor configuration, but also to the specific inoculum sources of the support material and this starting point can be crucial in carrying out the process in a long-term operation.

Inoculation is an important factor in bioreactors' start-up stage as the adoption of highly active inocula positively influences the imposed operational conditions in a shorter adaptation time and increases digestion efficiency (Neves et al. 2004). Moreno-Andrade and Buitrón (2004) studied the influence of the inoculum source on the anaerobic biodegradation of glucose and phenol, applying sludge from different treatment systems—a brewery wastewater treatment plant, an anaerobic reactor from the chemical industry, a municipal sewage treatment plant, an anaerobic digester of cow manure and activated sludge. The authors observed that the inoculum source influenced the glucose and phenol biodegradation, and the best biodegradation efficiency was achieved by using brewery sludge. Kim et al. (2017) studied the effects of three different inoculum sources on pharmaceutical and personal care products (PPCPs) biodegradation—activated sludge (AS), ditch sediment historically-impacted by WWTP effluent (Sd), and material from laboratory-scale soil aquifer treatment (SAT) columns. The authors found that the PPCPs removals were quite higher for AS and Sd (> 80% within 8 days), compared to the SAT inoculum (did not achieve partial results after twice the number of days for most of the analyzed substances) despite the comparable biomass, proving that the inoculum source affected the PPCP removal efficiency.

Given the potential for removing SMX during the wastewater biological treatment process presented by other research, this study aimed to assess the inoculum source influence on SMX biodegradation in an anaerobic structured bed biofilm reactor (ASBBR) using polyurethane foam as support media. Moreover, it was proposed to determine whether the choice of the inoculum for anaerobic reactors is a strategy to improve the start-up and the performance of biological technologies on the degradation of toxic compounds in a steady-state long-term operation, as well as influence the overall performance of the reactor in terms of the organic matter removal and biogas production.

Materials and methods

ASBBR configuration

The ASBBR consisted of a bench-scale tubular fixed bed bioreactor (total volume of 1.9 L) with an internal diameter of 6 cm, a reaction bed height of 50 cm and six intermediate

sampling points. The reactor was filled with polyurethane foam as support material, and consisted of eight prismatic strips of 55 cm length and 1 cm edge, arranged vertically in the reactor (Fig. S1—Supplementary Material). The fixed bed was supported using perforated stainless steel screens. The total useful volume was 1.6 L, resulting in a bed porosity of 84%. The water level inside the reactor was maintained by a water seal connected to the biogas meter, which controls the headspace level, allowing the accumulation of biogas in the headspace and escape by the upper part of the tube above the water level.

Reactor start-up and operating conditions

The ASBBR was operated with three different inoculum sources from full-scale UASB reactors applied to treat poultry slaughterhouse wastewater, brewery wastewater and domestic sewage, named: poultry slaughterhouse sludge (PS) (located in Pereira, São Paulo, Brazil), brewery sludge (BS) (located in Itu, São Paulo, Brazil), and sewage sludge (SS) (located in São Carlos, São Paulo, Brazil). Previous to the reactor operation, the support media was inoculated with sludge, adapting the methodology proposed by Zaiat et al. (1994). The procedure consisted of beating the granular sludge in a blender with water (40% water and 60% fresh sludge v/v). The foam remained in contact with the beaten sludge for a period of 2 h to promote the microorganism's adhesion. The reactor was operated at mesophilic conditions (30 °C) and the hydraulic retention time was set at 12 h.

The bioreactor operation with each inoculum went through an adaptation period to the substrate (described in Sect. 2.3) until reaching stable operating conditions in terms of COD (chemical oxygen demand) removal (> 70%) from the feed stream. After each adaptation period, SMX was applied in the feed stream and its removal was evaluated, as well as its impact on the anaerobic digestion.

Lab-made sewage composition

The ASBBR was fed continuously and in an upward flow with a laboratory-made domestic sewage, composed by 50% of protein (meat extract), 40% of carbohydrates (20% sucrose, 60% starch and 20% cellulose) and 10% of lipid (soybean oil emulsified with detergent solution)—organic loading rate of 0.4 kgCOD m^{-3} d^{-1}. The composition was adapted from Carneiro et al. (2019), as detailed in Table S1 (Supplementary Material). After being prepared, the feed solution was stored under refrigeration at 4 °C in a cold chamber to prevent its fermentation. SMX was added to the feed substrate at a concentration of around 400 ng L^{-1}. The pH of the feed solution was maintained at 7.4 ± 0.2 throughout the reactor operation by adding $NaHCO_3$ (300 mg L^{-1}).

Analytical methods and reactor performance monitoring

ASBBR performance monitoring was carried out by physical–chemical analysis in the influent and effluent samples in terms of pH, COD and solids content (total—TSS, fixed—FSS, and volatile suspended solids—VSS), according to the methods described in APHA (2005). Alkalinity (in $mgCaCO_3$ L^{-1}) was measured based on Ripley et al. (1986), and the ratio IA/PA (intermediate alkalinity/partial alkalinity) was also monitored in order to verify the occurrence of possible disturbances in the process, as the volatile fatty acids (VFA) are intermediate products of anaerobic digestion and an increase in their concentration might indicate an imbalance in this ratio. The VFA (acetic, propionic, butyric, isobutyric, valeric, isovaleric and caproic) were analyzed by gas chromatography (GC-2010 Shimadzu) based on Adorno et al. (2014).

The biogas composition, in terms of CH_4, CO_2 and H_2S, was analyzed on a gas chromatograph (Shimadzu GC 2014AT) equipped with a thermal conductivity detector (GC/TCD). The biogas flowrate (in mL d^{-1}) from the reactor was measured by the liquid displacement method, using a gasometer model MGC-1 V30 (Ritter). Taking into account the results of biogas composition and the flowrate, the following performance parameters of methanogenesis were determined: methane molar flow (*MMF*—in $mmolCH_4$ d^{-1}), volumetric methane production rate (*VMPR*—in $mLCH_4$ L^{-1} d^{-1}) and methane yield (*MY*—in $mLCH_4$ $g^{-1}COD_{removed}$), according to the Eqs. (1), (2) and (3). In these expressions, Q_{biogas} is the measured biogas flowrate (in mL d^{-1}); n_{CH4} is the number of methane moles (in µmol of CH_4) obtained by GC; V_i is the volume of biogas injected into the GC (500 µL of biogas); X_{CH4} is the CH_4 molar fraction (ratio methane moles/total biogas moles – in %); V_u is the useful volume of the reactor (in L); Q_{inf} is the reactor feed flowrate (in L d^{-1}); $COD_{inf} - COD_{eff}$ is the COD removed from the reactor by the methanogenic pathway (in gCOD L^{-1}).

$$MMF = \frac{Q_{biogas} \cdot n_{CH_4}}{V_i} \tag{1}$$

$$VMPR = \frac{Q_{biogas} \cdot X_{CH_4}}{V_u} \tag{2}$$

$$MY = \frac{Q_{biogas} \cdot X_{CH_4}}{Q_{inf}\left(COD_{inf} - COD_{eff}\right)} \tag{3}$$

The SMX quantification in the influent and effluent samples was determined following the procedure developed by Lima Gomes et al. (2015) and detailed by Carneiro et al. (2020), using an analysis system consisting of online solid

phase extraction (SPE), followed by high performance liquid chromatography (HPLC Agilent Technologies 1260 Infinity, USA) coupled with a hybrid triple quadrupole-linear ion trap mass spectrometer ABSciex QTrap 5500 (AB SCIEX, Foster, CA) equipped with an ESI source TurboV™ (SPE-HPLC-ESI-MS/MS), in a selected reaction monitoring mode. The sample preparation consisted of filtration at 0.22 µm, acidification with formic acid (98%) to pH 3.0, and addition of an isotopically labeled internal standard ($^{13}C_6$-SMX), in order to increase the detection accuracy of the method. The online SPE method allowed using an injection volume of 80 µL, pre-concentrating the samples around 200 times. The limits of detection and quantification obtained were 12.9 and 21.3 ng L^{-1}, respectively. SMX analysis in the biomass of the reactor bed was carried out at the end of each inoculum reactor operation to determine whether the sorption mechanism influenced the removal of SMX. The sorption assay protocol was according to Carneiro et al. (2019) and consisted of sequential extraction by adding separately methanol and acetonitrile (HPLC grade) in excess (8 mL) to the biomass (0.5 g of total suspended solids). Each solvent remained in contact with the biomass for 24 h at 30 °C and 150 rpm. After mixing both extracts, the final extract was dried and suspended in water (20 mL) for further analysis by LC–MS/MS, as previously described. The relative recovery reached in the analytical method was 99 ± 13%.

Statistical analyses of the monitoring and performance results of the ASBBR were performed in the PAST software (Hammer et al. 2001) applying one-way Analysis of Variance (ANOVA) of one factor and Tukey's pairwise test with a significance level (α) of 5%. The normal distribution of the experimental data was verified by Shapiro-Wilks tests. The samples were analyzed once (VFA and solids content) or twice (pH, COD, SMX, alkalinity and biogas composition) a week.

Biodegradation kinetics

To assess the COD and SMX biodegradation kinetics along the ASBBR reaction bed, samples were collected at the side points of the reactor (Fig. S1—Supplementary Material) under steady-state operation conditions. Each sampling point represented the different hydraulic retention times, ranging from 0 to 12 h. Taking into account that the ASBBR behaved as an ideal plug-flow reactor, as presented by Carneiro et al. (2019), the experimental data obtained along the bioreactor spatial profile were adjusted by a first-order kinetic model with residual concentration, as previously described by Camargo et al. (2002) and shown in Eq. (4). In this expression, C is the concentration (SMX or COD) in the liquid phase, C_0 is the influent concentration (SMX or COD), C_R is the residual concentration in the effluent stream (SMX or COD), HRT is the hydraulic retention time (in h), and k_1 is the first-order kinetic constant (in h^{-1}). The concentrations C_0 and C_R are in mg L^{-1} for COD and ng L^{-1} for SMX.

$$C = \left(C_0 - C_R\right).\ \exp\left(-k_1.HRT\right) + C_R \tag{4}$$

Microbiological analysis

The microbial communities' characterization of the inoculum and adhered biomass at the end of each operation was carried out using the PCR/DGGE (polymerase chain reaction/denaturing gradient gel electrophoresis) technique. The DNA extraction of those samples was performed with the FAST DNA Spin Kit for Soil kit (Bio101, Vista, CA, USA) according to the manufacturer's instructions. In the PCR step, fragments of the RNAr 16S gene were amplified using the 968 FGC—1401 R primer set for the Bacteria Domain and 1100 FGC—1400R for the Archaea Domain. DGGE was performed as described by Muyzer et al. (1993), using the DCode™ Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, CA, USA). The PCR product was separated by polyacrylamide gel electrophoresis containing a denaturing gradient of 45–65% (Muyzer et al. 1993). The DGGE running conditions were constant voltage of 75 V, at 60 °C for 16 h. The gels were observed in the Photodocumentation System (L.PixTouch—Loccus Biotechnology) under UV light exposure. From the DGGE band profile, BioNumerics software version 7.6.2 was used to calculate the similarity coefficient and dendrogram construct. The calculation of ecological indices—dominance, diversity (Shannon) and richness (Chao-1), was performed using the PAST software (Hammer et al. 2001).

To perform the microbiological assays, the inoculum and the adhered biomass in the support media at the end of each inoculum reactor operation were subjected to phase contrast and fluorescence microscopy using an optical microscope (LEICA), coupled to an OPTRONICS camera and IMAGE PRO-PLUS software. For the analysis, a drop of the sample was added to a slide, a thin layer of sterile agar (2%) was added and a cover slip was placed above the drop.

Results and discussion

ASBBR performance

The ASBBR operation lasted for 91, 100 and 108 days for PS, BS and SS inocula, respectively. The adaptation period without SMX in the feed stream in each operating condition was set as the period necessary to reach COD removal > 70% and CH_4 molar fraction > 90% in the biogas composition. These periods lasted seven days for the PS inoculum, ten days for the BS inoculum and one month

for the SS inoculum. Therefore, the inoculum source influenced the stabilization of the operational system during the start-up period of the reactor. In general, the full-scale anaerobic reactors need months in their start-up to reach a steady state operation in terms of organic matter removal efficiency (Escudié et al. 2011). Some authors (Priya et al. 2015; Rodriguez and Zaiat 2011; Zinatizadeh et al. 2017) also observed the efficiency of decreasing the duration of the reactors start-up period by applying immobilized biomass in fixed bed biofilm reactors.

After the period of biomass acclimatization, the influence of each inoculum source on the bioreactor performance was evaluated. Figure 1 shows the temporal variation of the influent, effluent and removal of COD and SMX for each operation. The bioreactor reached a quite high COD removal efficiency during the throughout operation ($86 \pm 3\%$, $84 \pm 4\%$ and $84 \pm 5\%$ for PS, BS and SS inocula, respectively). Comparing the results among the inocula by applying ANOVA and Tukey's post hoc test ($\alpha = 5\%$), it was concluded that the inoculum source did not influence

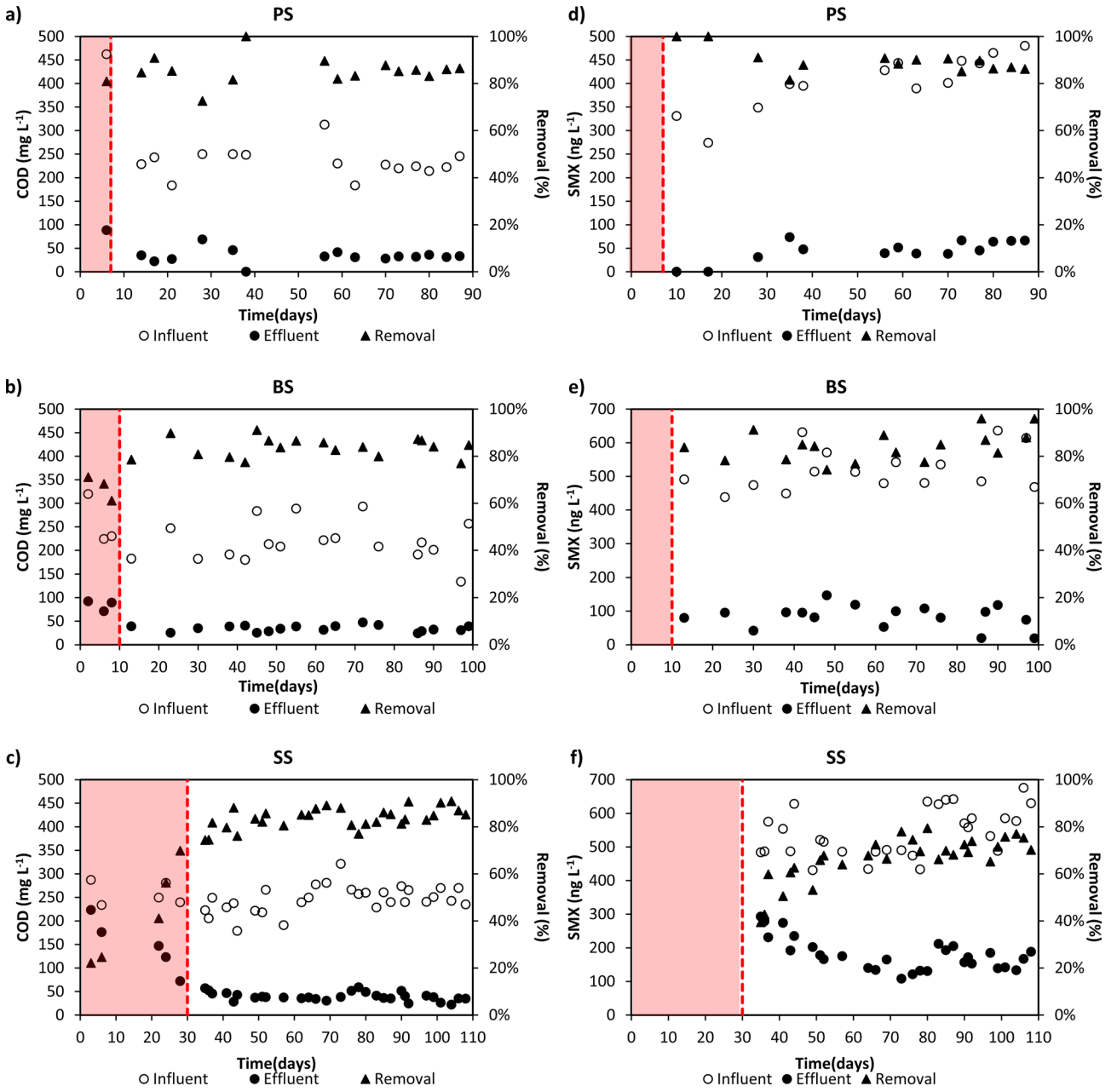

Fig. 1 Temporal variation of the influent, effluent and removal of COD (**a**–**c**) and SMX (**d**–**f**) for each reactor operation. The period in red is the adaptation time for each inoculum

the overall organic matter removal, which remained stable along the time.

The boxplot graphs of COD and SMX removal are presented in Fig. 2. PS, BS and SS inocula ASBBR operation led to SMX biodegradation of 90 ± 5%, 84 ± 6% and 70 ± 5%, respectively. These results also demonstrate the high stability of the operating system, as evidenced by the low standard deviation values obtained. Comparing the operation of each inoculum, significant differences (ANOVA test, α of 5%) in the removal efficiency were observed. The values of SMX removal with PS and BS inoculum are within the efficiencies found in other studies, e.g., Carneiro et al. (2019), who achieved 83 ± 12% of SMX removal in an ASBBR, and Chatila et al. (2016), who achieved 97% of SMX removal in a HAIB (horizontal-flow anaerobic immobilized biomass) reactor. These studies also applied poultry slaughterhouse (located in Tietê, São Paulo, Brazil) sludge as the inoculum source. The longer adaptation period of the SS biomass might also have contributed to the lower SMX biodegradation capacity during the SS-inoculum operation. Kang et al. (2018) found SMX removal of 84% and 73% in an anoxic/anaerobic/oxic granular and activated sludge reactor, respectively, and attributed the higher efficiency to the granules due to the greater retention and concentration of biomass, which is more effective in removing sulfonamide pharmaceuticals. The results showed that the presence of SMX did not harm the COD removal. In agreement with these results, previous studies indicated that sulfonamides do not have a negative impact on methanogenesis in typical sewage concentrations (ranges quite below mg L^{-1}), allowing high efficiencies for removing COD (> 80%) from the bioreactors (Carneiro et al. 2020a,b; Fountoulakis et al. 2004; Oliveira et al. 2017).

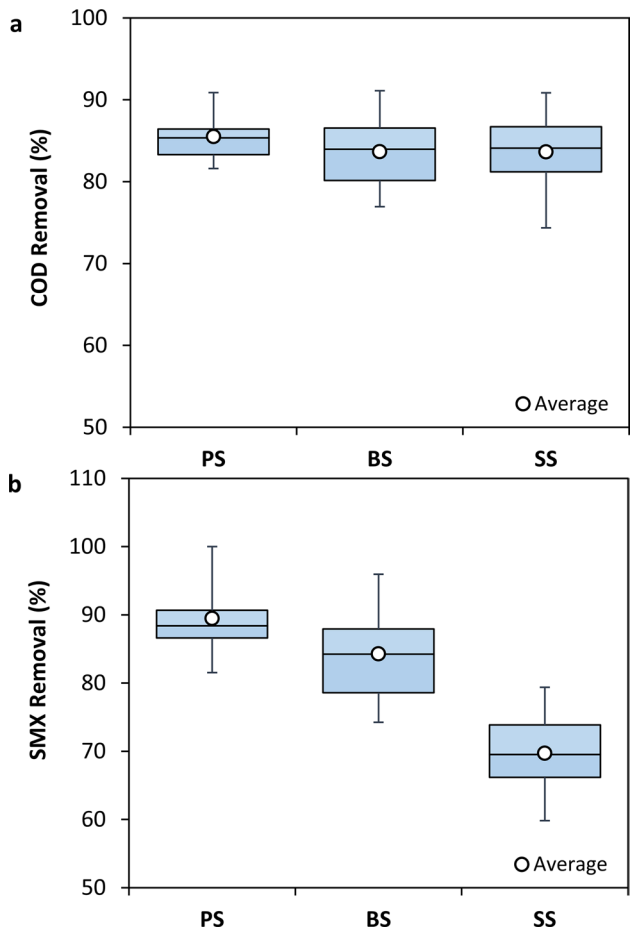

Fig. 2 Boxplot of COD (**a**) and SMX (**b**) removal in the bioreactor for each inoculum

The reactor reached quite a high CH_4 content in the biogas (> 90%), which remained stable throughout the operation. Figure 3 shows the boxplot of VMPR, MMF and MY in each inoculum reactor operation. The results showed that the different inocula did not lead to significant differences (α of 5%) in the biomethane yield in the reactor. According to Alvarino et al. (2014), the biodegradation of pharmaceutical compounds depends on the good methanogenic activity in anaerobic reactors, corroborating the high levels of SMX removal reached in this study. Li et al. (2011) studied the effect of inoculum source on anaerobic digestion performance and biogas production, and observed that the inoculum from the swine manure digester obtained better methane yield (212 $mLCH_4\ g^{-1}$ volatile solids) compared to the inocula from dairy manure, corn stover, and a municipal sludge digester, which presented a reduction from 8 to 11% in the methane yield. Li et al. (2013) evaluated the methane production during the digestion of organic substrates of chicken manure, using two different inocula—digested sludge from a municipal wastewater treatment plant (DSMW) and from a chicken manure-based anaerobic digester (DSCM), and found that DSMW presented better results regarding methane production (351 and 298 $mLCH_4\ g^{-1}$ volatile solid for DSMW and DSCM, respectively).

Comparing the MY results with those obtained by Carneiro et al. (2019), who applied poultry slaughterhouse sludge as inoculum source and obtained MY from 99 to 199 $mLCH_4\ g^{-1}COD_{removed}$ in an ASBBR of 2.7 L, the bioreactor operation with the three different inoculum sources (PS, BS and SS) reached higher results (Fig. 3c) in a reactor with a 61% smaller useful volume. This might indicate that the ASBBR reactor configuration can be very compact and still generate very satisfactory results in terms of organic matter removal and biomethane production.

By evaluating the biomass concentration at the end of each reactor operation (Fig. S2—Supplementary Material), the VSS values in the bed reactor corroborate that the PS inoculum operation performed better despite lower biomass content (72% less than the biomass amount in the BS operation). According to Delforno et al. (2017), the poultry slaughterhouse sludge presents a microbiota highly resistant

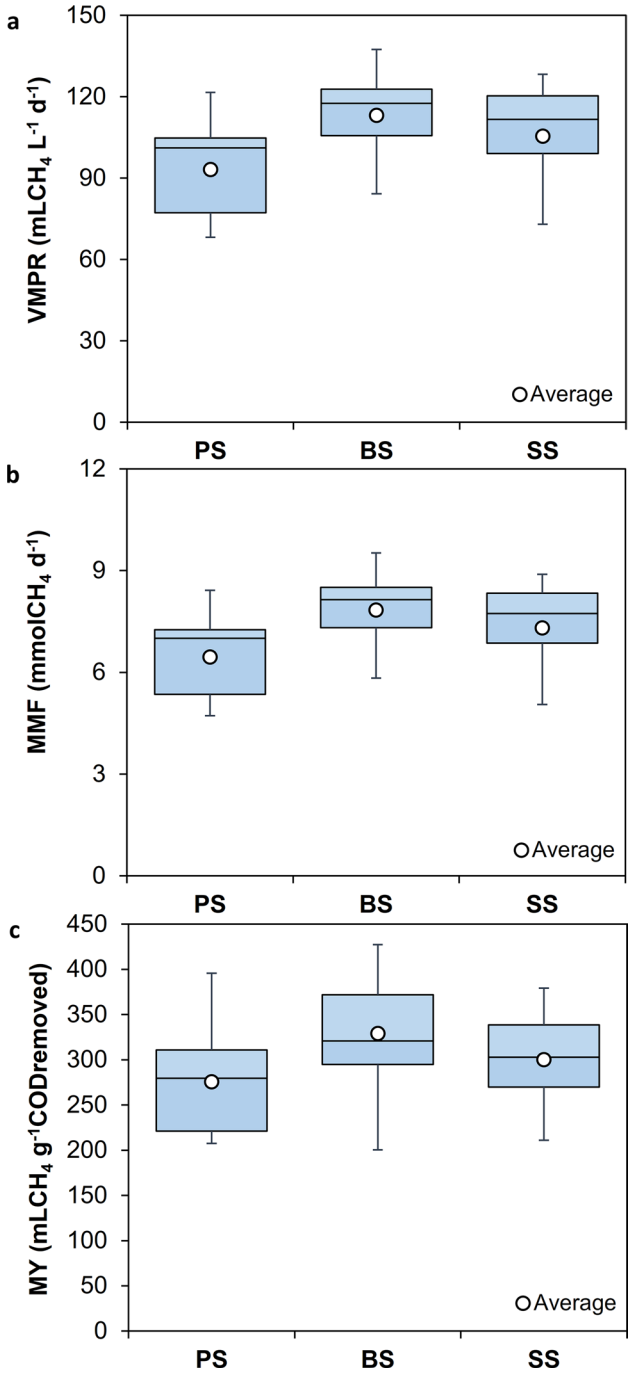

Fig. 3 Boxplot of volumetric methane production rate (**a**), methane molar flow (**b**) and methane yield (**c**) for each inoculum reactor operation

to antibiotics. These authors analyzed the broad spectrum profiles of the microbial composition and metabolic diversity of a full-scale UASB reactor inoculum applied to poultry slaughterhouse wastewater treatment and found that the microbial community contained 43 different types of antibiotic resistance genes, including sulfonamides, some of which are associated with promoting the growth of chickens (e.g., bacitracin, tetracycline and polymyxin). Further details of the reactor performance are summarized in Table S2 (Supplementary Material).

Biodegradation kinetics

Residual SMX in the biomass samples attached in the bed bioreactor support media after each inoculum reactor operation was not detected. Thus, the antibiotic removal was entirely due to the biodegradation, in agreement with previous studies (Alvarino et al. 2014; Carneiro et al. 2020). Likewise, Oliveira et al. (2017) demonstrated that a sorption mechanism was not significant for sulphamethazine (SMZ—an antibiotic belonging to the sulfonamide class) removal in a horizontal flow immobilized biomass reactor and found that the mechanisms of hydrolysis, photolysis, volatilization and adsorption to the reactor walls in removing SMZ were negligible. Moreover, the authors attribute these results to the quite low concentration in the feed stream and the low solid-water partition coefficient (K_D) of the sulfonamide (range of 13–72 L g^{-1}total solids).

Figure 4 shows the kinetic spatial profiles for COD and SMX removal. The first-order kinetic model adjusted quite well to the experimental data ($r^2 \geq 0.94$). The organic matter degradation during the PS and BS operation occurred mainly between the influent and the fifth sampling point (HRT of 9 h), indicating an excess capacity of the reactor, i.e., if there was an increase in the applied organic loading rate, it would not significantly affect the organic matter removal efficiency. Regarding SMX biodegradation, PS and BS operation led to a stabilization in the kinetic profile from the third sampling point (HRT of 5 h) and SS operation from the fifth point. Table 1 shows the adjustment parameters of the kinetic model for COD and SMX biodegradation. The first-order kinetic constants for COD were 0.40 ± 0.04 h^{-1}, 0.61 ± 0.14 h^{-1}, and 0.19 ± 0.02 h^{-1} and for SMX were 0.79 ± 0.35 h^{-1}, 1.24 ± 0.41 h^{-1}, and 0.57 ± 0.38 h^{-1} considering the PS, BS and SS operation, respectively. Thus, the BS-inoculum operation showed the more favorable kinetics for COD and SMX, while the SS-inoculum operation presented the lowest k_1, indicating that this sludge presented the lower capacity to biodegrade the antibiotic. In addition, the biodegradation kinetics seem to indicate a cometabolism in SMX biodegradation related to the COD removal, since the decay of the antibiotic along the bioreactor length followed the organic matter removal. Fischer and Majewsky (2014) stated that organic micropollutant biodegradation is likely driven by cometabolism, since the amounts of micropollutants are too low to serve as growth substrate. Gonzalez-Gil et al. (2017) investigated the mechanisms involved in the biodegradation of various organic micropollutants, including SMX, and proved that this occurs via an enzymatic

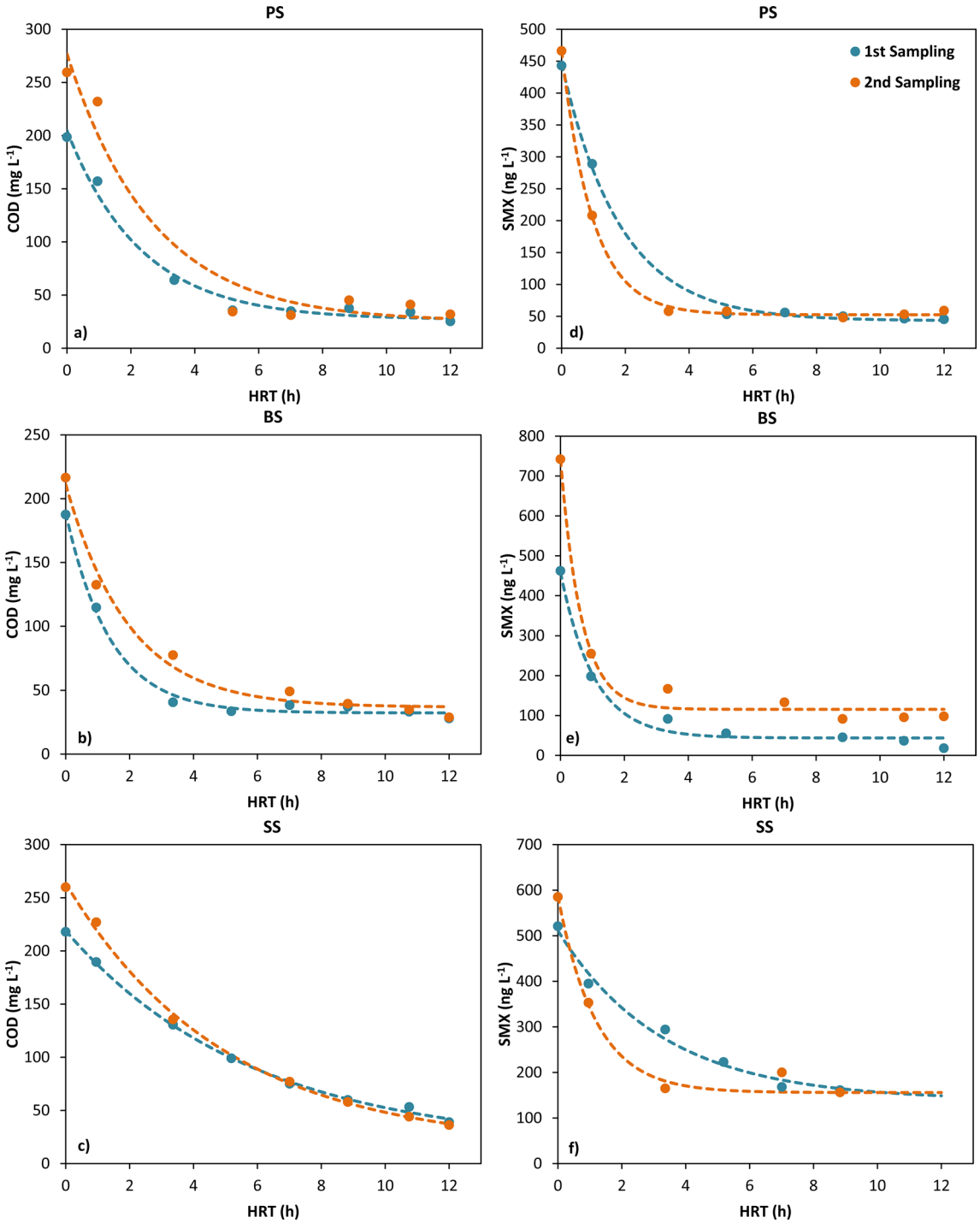

Fig. 4 Biodegradation kinetic profiles of COD (**a**–**c**) and SMX (**d**–f) removal and adjusted profiles for each inoculum reactor operation. The dotted lines indicate the adjustments obtained according to Eq. (4) by applying Origin software (version 8.5) and the points are the experimental data

cometabolic pathway in the biochemical process. Kennes-Veiga et al. (2021) recently showed that the SMX biodegradation kinetic constant presents a linear dependence with the organic loading rate, which also corroborates the hypothesis of cometabolic biodegradation of SMX in the ASBBR.

Microbial community

Comparing the microbial community present in the sludge before its inoculation and after its operation (residual sludge) through cluster analysis of the DGGE band profile using

Pearson's similarity coefficient (Fig. 5), significant changes were observed as the similarity for the Bacteria Domain was 74%, 9% and 9% and for the Archaea Domain 79%, 88% and 14% for PS-, BS- and SS-operation, respectively. These results indicate that the microbial community of poultry slaughterhouse and brewery sludge adapted better to the operating conditions in the reactor, considering both the Bacteria and Archaea domains, which corroborates the higher results in terms of SMX removal (PS—90 ± 5%; BS—84 ± 6%). Nonetheless, the SS inoculum presented significant changes in its microbial community after the ASBBR operation and proved to be more sensitive to the SMX presence, indicated by the lower similarity indices of Bacteria and Archaea domain compared to the other inocula, which is in agreement with the lower values showed in terms of SMX removal (70 ± 5%).

Table 2 shows the ecological indices for the Bacteria and Archaea domains. The Shannon indices refer to the

Table 1 First-order kinetic expressions estimated for COD and SMX biodegradation for each inoculum reactor operation

Parameter	1st sampling				2nd sampling			
	C_0	C_R	k_1 (h^{-1})	r^2	C_0	C_R	k_1 (h^{-1})	r^2
COD (PS)	205	27	0.43	0.982	277	25	0.37	0.943
COD (BS)	189	32	0.71	0.993	211	37	0.51	0.980
COD (SS)	219	16	0.17	0.998	264	16	0.20	0.997
SMX (PS)	446	43	0.54	0.997	466	52	1.03	0.997
SMX (BS)	459	44	0.95	0.981	741	115	1.53	0.982
SMX (SS)	511	139	0.30	0.970	587	156	0.84	0.973

Concentrations (C_0 and C_R) in mg L^{-1} for COD and ng L^{-1} for SMX

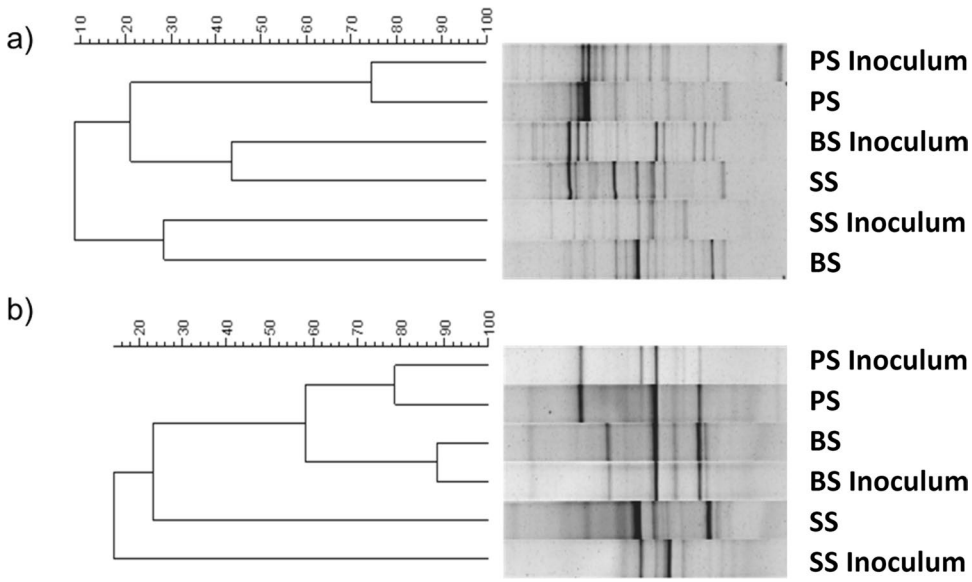

Fig. 5 Dendogram constructed based on Pearson's similarity coefficient from the DGGE band pattern for the Bacteria (**a**) and Archaea (**b**) domains of the inoculum and residual biomass after the ASBBR operation

Table 2 Alpha-diversity indices of microbial characterization of each inoculum and residual communities at the end of the ASBBR operation

Indice	PS Inoc	BS Inoc	SS Inoc	PS	BS	SS
Bacteria domain						
Dominance—D	0.10	0.09	0.11	0.12	0.14	0.12
Diversity—Shannon	2.54	2.65	2.37	2.48	2.32	2.39
Richness—Chao-1	17	21	13	19	15	15
Archaea domain						
Dominance—D	0.22	0.23	0.15	0.19	0.19	0.12
Diversity—Shannon	1.72	1.69	2.05	1.83	1.81	2.27
Richness—Chao-1	8	8	10	9	8	12

ecological diversity of the biomass samples. The values for the Bacteria domain decreased for all residual sludge samples compared to their respective inoculum biomass, while for the Archaea domain they increased for all residual samples. These results indicate that the methanogenic activity was favored during the ASBBR operation, which corroborates the quite high values of specific methane production and yield. According to Briones and Raskin (2003), the diversity of microorganisms tends to grow in a stressful environment. The higher Shannon indices of the Bacteria domain for PS after the ASBBR operation compared to the other inocula indicates a better balance between the hydrolytic/fermentative bacteria and the methanogenic archaea, which is in agreement with the better results in terms of COD and SMX removal, as previously pointed out. Moreover, the higher dominance indices of the methanogenic archaea community in PS and BS compared to SS (D indice: PS = 0.19; BS = 0.19; SS = 0.12) might also have influenced the greater SMX biodegradation observed during the bioreactor operation with PS and BS, since according to Cetecioglu et al. (2016) the biodegradation of SMX is driven by methanogenesis.

Microscopic analysis of the sludge after the ASBBR operation presented organisms similar to *Methanosaeta* in the PS and SS inocula, which indicate a methanogenic pathway, preferably acetoclastic in the reactor; and the presence of organisms similar to *Methanosaeta* and *Methanosarcina* in the BS inoculum, in addition to fluorescent bacilli—Fig. S3 (Supplementary Material). According to Fountoulakis et al. (2004), acetoclastic methanogens are the group most sensitive to SMX exposure of microorganisms that participate in anaerobic digestion. This reaffirms that the concentration of the compound to which the inocula were exposed caused small changes in the microbial communities (as observed by ecological indices), but it was not sufficient to inhibit the activity of these microorganisms. In addition, the results indicate that different microbial populations might play the same functional and metabolic role in the anaerobic cometabolic biodegradation of SMX in the reactor.

Conclusions

This study showed that the inoculum source played an important role in the SMX biodegradation during the anaerobic digestion but did not influence the anaerobic digestion in terms of COD removal and biomethane production, which remained quite stable throughout the operation for all inocula. The ASBBR proved to be efficient in removing COD (above 84%) and presented a high methane production (methane yield above 276 $mLCH_4\ g^{-1}COD_{removed}$) under an organic loading rate of 0.4 $kgCOD\ m^{-3}\ d^{-1}$ and hydraulic retention time of 12 h. These results indicate the establishment of functionally similar microbial communities in the reactor, but with different SMX biotransformation capabilities. The PS and BS inocula achieved better antibiotic removal (90 ± 5% and 84 ± 6%, respectively) than the SS inoculum (70 ± 5%). The kinetic assessment of COD and SMX decay indicated the occurrence of a cometabolic biodegradation of the sulfonamide. It was concluded from the molecular biology analysis that the SS inoculum was the most sensitive to the SMX presence after the bioreactor operation, considering both the Bacteria and Archaea domains, in agreement with the lower SMX removal compared to the other inocula.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s43153-021-00178-3.

Acknowledgements This work was supported by the São Paulo Research Foundation (FAPESP—processes nº 2017/18718-6, 2015/06246-7, 2015/04427-4 and 2009/53850-6) and the Coordination for the Improvement of Higher Education Personnel (CAPES—Finance Code 001). The authors would also like to thank Dr. Eloisa Pozzi and Dr. Maria Angela for their technical and scientific support.

Declarations

Conflict of interest No conflict of interest.

References

Adorno M, Hirasawa J, Varesche M (2014) Development and validation of two methods to quantify volatile acids. Am J Anal Chem 5(May):406–414. https://doi.org/10.4236/ajac.2014.57049

Alvarino T, Suarez S, Lema JM, Omil F (2014) Understanding the removal mechanisms of PPCPs and the influence of main technological parameters in anaerobic UASB and aerobic CAS reactors. J Hazard Mater 278:506–513. https://doi.org/10.1016/j.jhazmat.2014.06.031

American Public Health Association (APHA) (2005) Standard methods for the examination of water and wastewater. american water works association/American public works association/water environment federation, 21st edn. American Water Works Association/American Public Works Association/Water Environment Federation, Washington, DC, USA. https://doi.org/10.2105/AJPH.51.6.940-a

Briones A, Raskin L (2003) Diversity and dynamics of microbial communities in engineered environments and their implications for process stability. Curr Opin Biotechnol. https://doi.org/10.1016/S0958-1669(03)00065-X

Camargo EFM, Ratusznei SM, Rodrigues JAD, Zaiat M, Borzani W (2002) Treatment of low-strength wastewater using immobilized biomass in a sequencing batch external loop reactor: Influence of the medium superficial velocity on the stability and performance. Braz J Chem Eng 19(3):267–275. https://doi.org/10.1590/S0104-66322002000300001

Carneiro RB, Sabatini CA, Santos-Neto ÁJ, Zaiat M (2019) Feasibility of anaerobic packed and structured-bed reactors for sulfamethoxazole and ciprofloxacin removal from domestic sewage. Sci Total Environ 678:419–429. https://doi.org/10.1016/j.scitotenv.2019.04.437

Carneiro RB, Gonzalez-Gil L, Londoño YA, Zaiat M, Carballa M, Lema JM (2020a) Acidogenesis is a key step in the anaerobic biotransformation of organic micropollutants. J Hazard Mater 389:121888. https://doi.org/10.1016/j.jhazmat.2019.121888

Carneiro RB, Mukaeda CM, Sabatini CA, Santos-Neto ÁJ, Zaiat M (2020b) Influence of organic loading rate on ciprofloxacin and sulfamethoxazole biodegradation in anaerobic fixed bed biofilm reactors. J Environ Manag. https://doi.org/10.1016/j.jenvman.2020.111170

Cerreta G, Roccamante MA, Plaza-Bolaños P, Oller I, Aguera A, Malato S, Rizzo L (2020) Advanced treatment of urban wastewater by UV-C/free chlorine process: micro-pollutants removal and effect of UV-C radiation on trihalomethanes formation. Water Res. https://doi.org/10.1016/j.watres.2019.115220

Cetecioglu Z, Ince B, Orhon D, Ince O (2016) Anaerobic sulfamethoxazole degradation is driven by homoacetogenesis coupled with hydrogenotrophic methanogenesis. Water Res 90:79–89. https://doi.org/10.1016/j.watres.2015.12.013

Chatila S, Amparo MR, Carvalho LS, Penteado ED, Tomita IN, Santos-Neto ÁJ et al (2016) Sulfamethoxazole and ciprofloxacin removal using a horizontal-flow anaerobic immobilized biomass reactor. Environ Technol (united Kingdom) 37(7):847–853. https://doi.org/10.1080/09593330.2015.1088072

Chernicharo CAL, van Lier JB, Noyola A, Bressani Ribeiro T (2015) Anaerobic sewage treatment: state of the art, constraints and challenges. Rev Environ Sci Biotechnol 14(4):649–679. https://doi.org/10.1007/s11157-015-9377-3

Danner M, Robertson A, Behrends V, Reiss J (2019) Antibiotic pollution in surface fresh waters : Occurrence and effects. Sci Total Environ 664:793–804. https://doi.org/10.1016/j.scitotenv.2019.01.406

Delforno TP, Lacerda GV, Noronha MF, Sakamoto IK, Varesche MBA, Oliveira VM (2017) Microbial diversity of a full-scale UASB reactor applied to poultry slaughterhouse wastewater treatment: Integration of 16S rRNA gene amplicon and shotgun metagenomic sequencing. Microbiol Open 6:e443.https://doi.org/10.1002/mbo3.443

Du L, Xu W, Liu Y, Li X, Huang D, Wu S (2020) Removal of sulfamethoxazole in aqueous solutions by iron-based advanced oxidation processes: performances and mechanisms. Water Air Soil Pollut. https://doi.org/10.1007/s11270-020-04534-w

Escudié R, Cresson R, Delgenès JP, Bernet N (2011) Control of start-up and operation of anaerobic biofilm reactors: an overview of 15 years of research. Water Res 45(1):1–10. https://doi.org/10.1016/j.watres.2010.07.081

Fischer K, Majewsky M (2014) Cometabolic degradation of organic wastewater micropollutants by activated sludge and sludge-inherent microorganisms. Appl Microbiol Biotechnol 98(15):6583–6597. https://doi.org/10.1007/s00253-014-5826-0

Fountoulakis M, Drilla P, Stamatelatou K, Lyberatos G (2004) Toxic effect of pharmaceuticals on methanogenesis. Water Sci Technol 50(5):335–340. https://doi.org/10.2166/wst.2004.0346

Gonzalez-Gil L, Carballa M, Lema JM (2017) Cometabolic enzymatic transformation of organic micropollutants under methanogenic conditions. Environ Sci Technol 51(5):2963–2971. https://doi.org/10.1021/acs.est.6b05549

Grenni P, Ancona V, Barra Caracciolo A (2018) Ecological effects of antibiotics on natural ecosystems: a review. Microchem J 136:25–39. https://doi.org/10.1016/j.microc.2017.02.006

Gworek B, Kijeńska M, Wrzosek J, Graniewska M (2021) Pharmaceuticals in the soil and plant environment: a review. Water Air Soil Pollut. https://doi.org/10.1007/s11270-020-04954-8

Hammer Ø, Harper DAT, Ryan PD (2001) PAST: paleontological statistics software package for educatioon and data analysis. https://palaeo-electronica.org/2001_1/past/issue1_01.htm

Kang AJ, Brown AK, Wong CS, Yuan Q (2018) Removal of antibiotic sulfamethoxazole by anoxic/anaerobic/oxic granular and suspended activated sludge processes. Biores Technol. https://doi.org/10.1016/j.biortech.2017.12.021

Kennes-Veiga DM, Gonzalez-Gil L, Carballa M, Lema JM (2021) The organic loading rate affects organic micropollutants' cometabolic biotransformation kinetics under heterotrophic conditions in activated sludge. Water Res 189:116587. https://doi.org/10.1016/j.watres.2020.116587

Kim S, Rossmassler K, Broeckling CD, Galloway S, Prenni J, De Long SK (2017) Impact of inoculum sources on biotransformation of pharmaceuticals and personal care products. Water Res 125:227–236. https://doi.org/10.1016/j.watres.2017.08.041

Langbehn RK, Michels C, Soares HM (2021) Antibiotics in wastewater: from its occurrence to the biological removal by environmentally conscious technologies. Environ Pollut 275:116603. https://doi.org/10.1016/j.envpol.2021.116603

Li L, Yang X, Li X, Zheng M, Chen J, Zhang Z (2011) The influence of inoculum sources on anaerobic biogasification of NaOH-treated corn stover. Energy Sources Part a: Recovery Util Environ Effects 33(2):138–144. https://doi.org/10.1080/15567030902937192

Li Y, Feng L, Zhang R, He Y, Liu X, Xiao X et al (2013) Influence of inoculum source and pre-incubation on bio-methane potential of chicken manure and corn stover. Appl Biochem Biotechnol 171(1):117–127. https://doi.org/10.1007/s12010-013-0335-7

Lima Gomes PCF, Tomita IN, Santos-Neto ÁJ, Zaiat M (2015) Rapid determination of 12 antibiotics and caffeine in sewage and bioreactor effluent by online column-switching liquid chromatography/tandem mass spectrometry. Anal Bioanal Chem 407(29):8787–8801. https://doi.org/10.1007/s00216-015-9038-y

Liu S, Hassan SU, Ding H, Li S, Jin F, Miao Z et al (2020) Removal of sulfamethoxazole in water by electro-enhanced Co2+/peroxydisulfate system with activated carbon fiber-cathode. Chemosphere 245:125644. https://doi.org/10.1016/j.chemosphere.2019.125644

Monteoliva-García A, Martín-Pascual J, Muñío MM, Poyatos JM (2020) Effects of carrier addition on water quality and pharmaceutical removal capacity of a membrane bioreactor—advanced oxidation process combined treatment. Sci Total Environ. https://doi.org/10.1016/j.scitotenv.2019.135104

Moreno-Andrade I, Buitrón G (2004) Influence of the origin of the inoculum on the anaerobic biodegradability test. Water Sci Technol 49(1):53–59. https://doi.org/10.2166/wst.2004.0017

Muyzer G, de Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol 59(3):695–700. https://doi.org/10.1128/aem.59.3.695-700.1993

Neves L, Oliveira R, Alves MM (2004) Influence of inoculum activity on the bio-methanization of a kitchen waste under different waste/inoculum ratios. Process Biochem 39(12):2019–2024. https://doi.org/10.1016/j.procbio.2003.10.002

Oliveira GHD, Santos-Neto AJ, Zaiat M (2017) Removal of the veterinary antimicrobial sulfamethazine in a horizontal-flow subjectan-aerobic immobilized biomass (HAIB) reactor ed to step changes in the applied organic loading rate. J Environ Manag 204:674–683. https://doi.org/10.1016/j.jenvman.2017.09.048

Park J, Kim C, Hong Y, Lee W, Chung H, Jeong DH, Kim H (2020) Distribution and removal of pharmaceuticals in liquid and solid phases in the unit processes of sewage treatment plants. Int J Environ Res Public Health. https://doi.org/10.3390/ijerph17030687

Priya M, Meenambal T, Balasubramanian N, Perumal B (2015) Comparative study of treatment of sago wastewater using HUASB reactor in the presence and absence of effective microorganisms. Procedia Earth Planet Sci 11:483–490. https://doi.org/10.1016/j.proeps.2015.06.048

Reis AC, Kolvenbach BA, Nunes OC, Corvini PFX (2020) Biodegradation of antibiotics: the new resistance determinants—part I. New Biotechnol 54:34–51. https://doi.org/10.1016/j.nbt.2019.08.002

Ripley LE, Boyle WC, Converse JC (1986) Improved alkalimetric-monitoring for anaerobic digestion of high-Strenght wastes. Water Pollut Control Fed. https://doi.org/10.1109/APMC.2005.1606770

Rodriguez RP, Zaiat M (2011) Influence of carbon source and inoculum type on anaerobic biomass adhesion on polyurethane foam in reactors fed with acid mine drainage. Biores Technol 102(8):5060–5065. https://doi.org/10.1016/j.biortech.2011.01.084

Shahmahdi N, Dehghanzadeh R, Aslani H, Bakht Shokouhi S (2020) Performance evaluation of waste iron shavings (Fe0) for catalytic ozonation in removal of sulfamethoxazole from municipal wastewater treatment plant effluent in a batch mode pilot plant. Chem Eng J 383:123093. https://doi.org/10.1016/j.cej.2019.123093

Singh R, Singh AP, Kumar S, Giri BS, Kim KH (2019) Antibiotic resistance in major rivers in the world: a systematic review on occurrence, emergence, and management strategies. J Clean Prod 234:1484–1505. https://doi.org/10.1016/j.jclepro.2019.06.243

Su D, Ben W, Strobel BW, Qiang Z (2021) Impacts of wastewater treatment plant upgrades on the distribution and risks of pharmaceuticals in receiving rivers. J Hazard Mater. https://doi.org/10.1016/j.jhazmat.2020.124331

Van Boeckel TP, Gandra S, Ashok A, Caudron Q, Grenfell BT, Levin SA, Laxminarayan R (2014) Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. Lancet Infect Dis 14(8):742–750. https://doi.org/10.1016/S1473-3099(14)70780-7

Wang S, Wang J (2018) Degradation of emerging contaminants by acclimated activated sludge. Environ Technol (united Kingdom) 39(15):1985–1993. https://doi.org/10.1080/09593330.2017.1345989

Yun MK, Wu Y, Li Z, Zhao Y, Waddell MB, Ferreira AM et al (2012) Catalysis and sulfa drug resistance in dihydropteroate synthase. Science. https://doi.org/10.7551/mitpress/8876.003.0036

Zaiat M, Cabral AKA, Foresti E (1994) Reator anaeróbio horizontal de leito fixo para tratamento de águas residuárias: concepção e avaliação preliminar de desempenho. Rev Bras Eng Quím 11(2):33–42

Zhao W, Sui Q, Mei X, Cheng X (2018) Efficient elimination of sulfonamides by an anaerobic/anoxic/oxic-membrane bioreactor process: performance and influence of redox condition. Sci Total Environ. https://doi.org/10.1016/j.scitotenv.2018.03.207

Zinatizadeh AA, Mohammadi P, Mirghorayshi M, Ibrahim S, Younesi H, Mohamed AR (2017) An anaerobic hybrid bioreactor of granular and immobilized biomass for anaerobic digestion (AD) and dark fermentation (DF) of palm oil mill effluent: mass transfer evaluation in granular sludge and role of internal packing. Biomass Bioenerg 103:1–10. https://doi.org/10.1016/j.biombioe.2017.05.006