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Evaluation of triclosan toxic effects on the methanogenic activity

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ABSTRACT

Background: Triclosan (TCS) is an antimicrobial agent widely used in health care and consumer products. This compound is present in sludge of wastewater treatment plants (WWTPs), and because of its bactericidal characteristics, it can inhibit the methanogenic activity in anaerobic digestion (AD) technology. The aim of this study was to evaluate the toxic effects of TCS on the methanogenic activity.
Results: Batch anaerobic reactors were used with TCS concentrations of 7.8, 15.7, 23.5, and 31.4 mg/L. These assays consisted in three successive feedings (I, II, and III), wherein the sludge was exposed to each TCS concentration and volatile fatty acid (VFA) substrate. For evaluation of the residual sludge activity during feeding III, only VFA was used. The results showed that the increase in TCS concentrations correlated with the reduction in methane (CH_4) production. In this case, the minimum values were achieved for TCS concentration of 31.4 mg/L with CH_4 levels between 101.9 and 245.3 during feedings I, II, and III. Regarding the effect of TCS on VFA consumption, an inhibitory effect was detected for TCS concentrations of 23.5 and 31.4 mg/L, with concentrations of acetic, butyric, and propionic acids at the end of the assay (37 d) between 153.6 and 206.8, 62.5 and 60.1, and 93.4 and 110 mg/L, respectively. Regarding the removal of TCS during AD, these values were above 47%.
Conclusion: TCS is an inhibitor of methanogenic activity with a decrease between 63 and 70% during the different feedings. The CH_4 production was not recovered during feeding III, with inhibition percentages of 21–72%.
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1. Introduction

Anaerobic digestion (AD) is a complex biological process widely used for sludge stabilization [1]. This technology is recognized for biogas production, low-energy consumption, and low sludge production, which is often disposed for agricultural purposes [2]. The final step of AD is methanogenesis, which consists in the formation of methane (CH_4) mediated by methanogenic archaea. The structure of the methanogenic community is very important for CH_4 production. In fact, the methanogens are the most sensitive microorganisms of AD and the presence of organic and inorganic compounds can potentially inhibit this process [3].

The inhibition of AD in the presence of micropollutants was related to some surfactants and pharmaceutical compounds [4]. The effects of sulfamethoxazole, erythromycin, and tetracycline on CH_4 concentrations were studied. With concentrations above 500 mg/L of these antimicrobial agents, the CH_4 production was completely inhibited [5]. Another antibacterial agent most commonly used is triclosan (2,4,4–trichloro-2-hydroxydiphenyl ether, TCS) [6]. This hydrophobic compound is used in health care and consumer products such as toothpastes, hand disinfectant soaps, and medical skin creams [7]. TCS is widely ubiquitous in wastewater treatment plants (WWTPs) with concentrations varying between 0.8 and 80 mg/kg of dry matter [4]. Because the logarithm of the octanol–water partition coefficient (Log K_{ow}) is above 4, this antimicrobial agent is mainly sorbed by biosolids, and consequently, it is not eliminated during the AD, thereby achieving a removal efficiency below 20% [8,9].

The effects of TCS on CH_4 production during the AD have been poorly documented. Recently, it has been reported that the presence of TCS can alter the anaerobic community composition, and consequently, it can inhibit the methanogenic activity of AD [10]. McNamara et al. [11] studied the effects of this antibacterial agent on the structure and function of anaerobic microbial communities. In this study, TCS concentrations used were 5, 50, and 500 mg/kg. The results showed that high-dose TCS (500 mg/kg) has a cumulative effect of 56% on CH_4 production compared to that of 123% produced by low-dose TCS (5 mg/kg). The same tendency was observed in Carey et al. [8], with TCS concentrations of 100, 850, and 2500 mg/kg. In this case, the CH_4

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production remained between 50 and 75 mL/d for TCS concentrations of 100 and 850 mg/kg. However, the CH_4 production decreased by 80% in digesters with a higher TCS concentration (2500 mg/kg). Similarly, Symsaris et al. [3] investigated the effect of different TCS concentrations and the impact of the addition of biomass on AD process efficiency using two methanogenic inocula: mesophilic WTTP sludge-based and a thermophilic manure-based inoculum. The results obtained in this study showed a correlation between increasing TCS levels of 20–320 mg/L and reduction in CH_4 production from approximately 180 mL CH_4/g·volatile solids (VS) to 6 mL CH4/g·VS. In the same study, the biomass addition during different assays reduced significantly the inhibition effect of TCS on AD process. For TCS concentration of 80 mg/L, the inhibition was reduced from 90% to 10%. Likewise, lack of information about the effects of TCS on volatile fatty acids (VFA) consumption has been reported. For evaluating the activity of anaerobic microorganisms, the presence of different VFAs is necessary. Stone et al. [17] studied the effects of tylosin and chlortetracycline on VFA consumption, including acetate, butyrate, and propionate, in biosolids from swine manure. The results of this study showed that VFA accumulation was greater for chlortetracycline than for tylosin with values of 12,269 mg/L.

Taking the above into account, the aim of this study was to evaluate the TCS toxic effects on the methanogenic activity by emphasizing the effects of this antibacterial compound on VFA consumption.

2. Material and methods

2.1. Inoculum

The anaerobic biomass is a granular sludge type derived from an anaerobic treatment system of a brewery. Regarding the physicochemical characteristics of the inoculum used in these experiments, pH, volatile suspended solids (VSS), and total suspended solids (TSS) values were 7.4, 23.4 mg/L, and 41.2 mg/L, respectively. The initial methanogenic activity of the sludge was 0.23 ± 0.067 g chemical oxygen demand (COD) $_{CH4}$/VSS·d.

2.2. Batch experimental setup

The methanogenic toxicity assays were performed following the methodology previously described by Belmonte et al. [12] and Reyes-Contreras and Vidal [13]. The assays were realized using a mixture of VFA and TCS as the toxic compound to be evaluated. For batch reactor experiment, 125 mL amber glass serum bottles were used. In each reactor, the total concentration of VFA added was composed of 2 g/L of acetic acid, 0.5 g/L of propionic acid, and 0.5 g/L of *n*-butyric acid (total COD from VFA was 3.8 g COD-VFA/L). The VFA solution was previously neutralized (pH: 7) with NaOH. The media also contained the following nutrients: NH_4Cl (0.14 g/L), K_2HPO_4 (0.125 g/L), $MgSO_4 \times 7H_2O$ (0.10 g/L), $CaCl_2 \times 2H_2O$ (0.01 g/L), and $NaHCO_3$ (0.2 g/L). Moreover, the inoculum concentration added to each reactor was 2 g VSS/L. $Na_2S \times 9H_2O$ (100 mg/L) was also added to generate the anaerobic conditions. Each reactor was sealed and bubbled with nitrogen gas (N_2) for 2 min to remove air from the headspace. Finally, samples were incubated at 35°C throughout the experiment. To determine CH_4 production, volumetric displacement method was carried out. This method is based on quantifying the amount of CH_4 using a displacing substance such as NaOH (2.5%), which reacts with the biogas precipitating the CO_2.

Three successive feedings, with each TCS concentration evaluated, were performed. In this case, the concentrations used were 0 (control), 7.8, 15.7, 23.5, and 31.4 mg/L. These concentrations tested were based on the study of Symsaris et al. [3], which used levels of TCS between 20 and 380 mg/L for evaluating the effect of TCS on methanogenic activity of mesophilic and thermophilic inoculum according to previous preliminary screening of the biomass. In the first feeding (I), the sludge was exposed to media containing TCS and VFA substrate. At the end of feeding I, the supernatant (spent medium) was carefully decanted and the sludge was again exposed to TCS and VFA substrate (feeding II). At the end of feeding II, the spent medium was removed. Finally, to evaluate residual sludge activity after the first and second exposures, a third feeding (III) was realized, which contained only the VFA mix solution as the substrate. The assays were carried out 35°C and incubated for 39 d. All assays were conducted in triplicate. The liquid fraction (supernatant) obtained for each reactor after each feeding was stored and subsequently monitored.

2.3. Analytical methods

2.3.1. In situ and physicochemical parameters

The in situ parameters electrical conductivity (EC), oxidation–reduction potential (ORP), and pH were measured using a multiparametric OAKTON-PC650 (Eutech Instruments, Singapore). For physicochemical characterization of the inoculum and the supernatant, COD, TSS, and VSS were determined according to the methodologies established in Standard Methods, specifically through the following procedures: 5220-C method for COD as well as 2540-D and 2540-E methods for TSS and VSS, respectively [14].

2.3.2. HPLC determination

For determining the concentrations of TCS in the supernatant, HPLC analysis was performed using a Shimadzu Prominence Liquid Chromatograph equipped with a UV detector (SPD-20 V Prominence UV/VIS) and an autosampler (SIL-20AC Prominence Autosampler) (Shimadzu, Japan). Data acquisition and processing were carried out using Shimadzu's LC solution software (Shimadzu, Japan). Chromatographic separations were performed on a HIQ Sil C18-HS (150 × 4.6 mm; KYATECH Corporation, Japan) at 30°C. The detector was set at a wavelength of 282 nm. The mobile phase consisted in acetonitrile:water (80:20, v/v); the flow rate was 1.0 mL/min, and the injection volume was 10 μL.

2.3.3. VFA determination

VFA was determined by gas chromatography (GC) (Shimadzu GC-2014, Kyoto, Japan) equipped with an autosampler (Shimadzu AOC 20i, Kyoto, Japan) and a flame ionization detector (FID), fitted with a 30 m × 0.32 mm I.D. × 0.25 μm thickness film Stabilwax-DA column (Restek Corporation; Bellefonte, PA, USA). The carrier gas was N_2 (purity 99.999%) at a constant flow rate of 2.23 mL/min. The oven temperature was held at 95°C for 1 min, then temperature programmed at 10°C/10 min until 140°C, and finally held for 5 min. A volume of 1 μL of sample was injected in the split mode at an injector temperature of 270°C. The FID temperature was 250°C. The chromatograms obtained were analyzed by GC Solution software, version 2.41 00SU1 (Shimadzu; Kyoto, Japan) [15].

3. Results and discussion

3.1. Effects of TCS on CH_4 production

Fig. 1 shows the daily production of CH_4 for each TCS concentration during feedings I, II, and III. The maximum CH_4 production was achieved for TCS concentration of 7.8 mg/L (Fig. 1a). In this case, the values obtained were 289.6 ± 11.52, 194.43 ± 11.37, and 142.0 ± 9.79 mL for feedings I, II, and III, respectively. In contrast, the minimum CH_4 production was observed for TCS concentration of 31.4 mg/L, with CH_4 levels of 245.35 ± 33.15, 130.70 ± 2.26, and 101.88 ± 10.32 mL for feedings I, II, and III, respectively (Fig. 1d). These results showed a correlation between the increase in TCS concentrations and the reduction in CH_4 production. The same tendency was reported for mesophilic WTTP sludge-based and thermophilic manure-based inoculum increasing TCS concentrations to 20, 80, 160, and 320 mg/L [3].

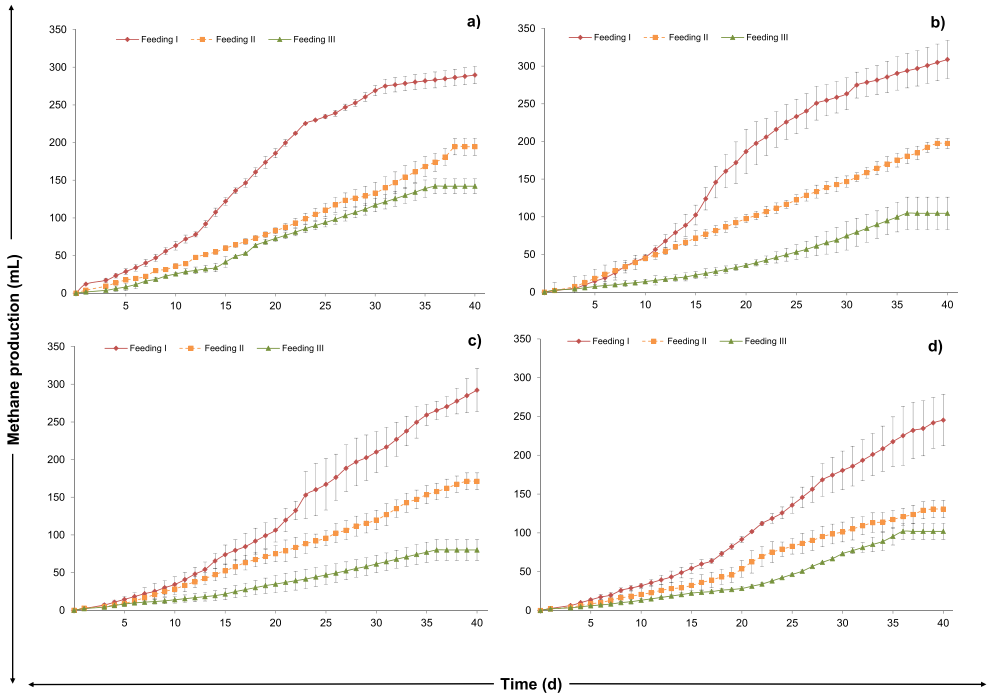

Fig. 1. Methane production (mL) accumulated in the methanogenic toxicity assays for different TCS concentrations (mg/L): (a) 7.8, (b) 15.7, (c) 23.5, and (d) 31.4 during feedings I, II, and III.

Regarding the evolution of CH_4 production during feeding I, it was stable until day 8 of incubation and reached a value of 46.9 mL for TCS concentration of 7.8 mg/L. However, for TCS concentrations of 15.7, 23.5, and 31.4 mg/L, the CH_4 productions were below 50 mL until days 11, 13, and 15, respectively. A possible explanation for these differences might be attributed to the acclimation process of methanogenic communities that may adapt to the TCS concentrations [11]. During days 10–36, the maximum CH_4 production was achieved with values ranging from 63.10 ± 5.84 to 283 ± 13 mL.

Fig. 2 shows the methanogenic activities under different TCS concentrations for each feeding. For all TCS concentrations evaluated (7.8, 15.7, 23.5, and 31.4 mg/L), the methanogenic activity decreased between 33 and 45% from feeding I to feeding II. In the case of feeding III, the methanogenic activity was between 63 and 70% lower than that feeding I. This behavior evidenced a progressive inhibition of methanogenic activity during feedings I, II, and III. In this case, the inhibition percentages increased between 21 and 72% from feeding I to feeding III. Particularly, for TCS concentrations of 7.8 and 15.7 mg/L, the inhibition was 72% and 51% higher in feeding III than in feeding I, respectively. Despite the use of VFA as the substrate, the methanogenic activity was not recovered during feeding III. A contrasting effect was observed in Symsaris et al. [3], where the inhibition effects of TCS were significantly reduced from 90 to 10% for TCS concentration of 80 mg/L during different feedings. In this case, sterile biomass was added to the two inoculums during the three feedings. These results showed that sorption capacity from the additional biomass reduced the TCS bioavailability counteracting its inhibition effect on AD. In fact, as mentioned previously, this compound has a log K_{ow} above 4, which indicated that TCS is mainly sorbed by the biosolids [8]. Thus, it seems that the inhibition effects of TCS on methanogenic activity is buffered by the biosolids absorption of TCS [3].

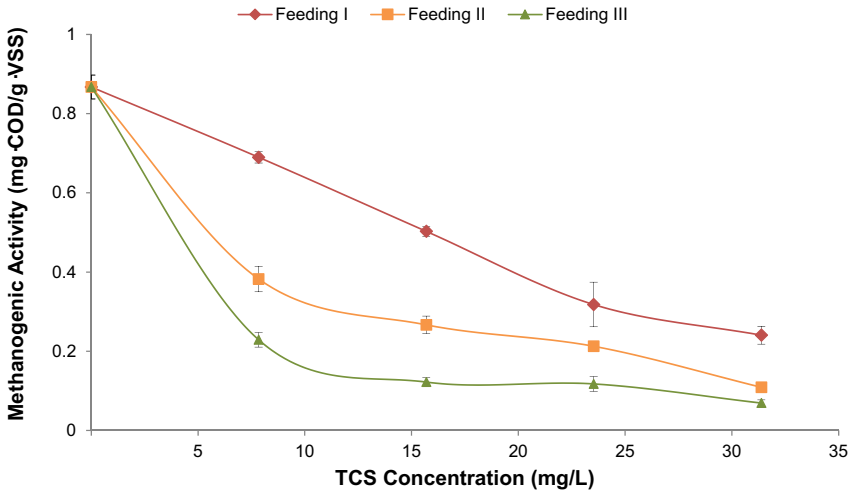

Fig. 2. Methanogenic activities (mg COD/g SSV·d) under different TCS concentrations (mg/L) for each feeding: I, II, and III.

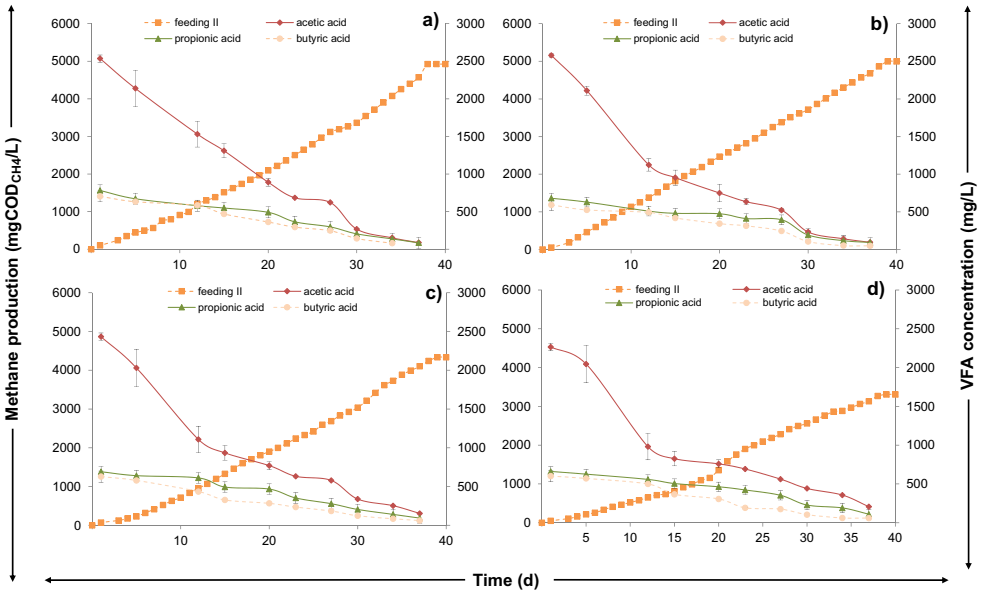

Fig. 3. Cumulative methane production (mg COD_{CH4}/L) in methanogenic toxicity assay and the VFA concentration (mg/L; acetic acid, propionic acid, and butyric acid) in the supernatant obtained from each reactor evaluated in the second feeding, considering the different TCS concentrations (mg/L) evaluated: (a) 7.8, (b) 15.7, (c) 23.5, and (d) 31.4.

3.2. Effects of TCS on VFA consumption

Fig. 3 shows the cumulative CH_4 production versus the kinetics of VFA transformation during feeding II considering the different TCS concentrations evaluated. VFA concentrations are central for evaluating the performance of AD [16]. The acetic, propionic, and butyric acid concentrations were evaluated on days 1, 5, 12, 15, 20, 23, 27, 30, 34, and 37, with initial average values (1 d) of 2533.6 ± 34.6, 781.3 ± 21.7, and 702.3 ± 15.6 mg/L, respectively. For all TCS concentrations, there exists consumption of VFA by methanogenic microorganisms present in reactors. As shown Fig. 3a and Fig. 3b, an inhibitory effect of TCS was not observed for TCS concentrations of 7.8 and 15.7 mg/L. In these cases, only acetic acid was accumulated on 37 d, with concentrations of 87.43 ± 10.14 and 95.53 mg/L, respectively. However, an inhibitory effect was detected for TCS concentrations of 23.5 and 31.4 mg/L. The concentrations of acetic, butyric, and propionic acids at the end of the assay (37 d) were between 153.6 and 206.8, 62.5 and 60.1, and 93.4 and 110 mg/L. These results suggested that the efficiencies of methanogenesis were directly affected by the TCS concentrations. Moreover, these acetate levels indicated that the utilization of acetate by homoacetogenic bacteria or aceticlastic methanogens was inhibited [17]. This behavior was similar to that reported by other antimicrobial compounds that inhibited the AD process, such as propranolol hydrochloride, ofloxacin, diclofenac sodium, chlortetracycline hydrochloride, sulfamethoxazole-tetracycline, erythromycin-sulfamethoxazole, erythromycin-tetracycline, and erythromycin-tetracycline-sulfamethoxazole [2,13,18].

Regarding the negative effects of TCS on the CH_4 production and VFA consumption during AD, this compound is toxic for the methanogenic activity. The mechanisms of TCS toxicity have been studied recently, and they are related to the changes in the microbial community composition. Multiple species have developed resistance to TCS because this compound has the capacity to inhibit fatty acid synthesis in cells at low concentrations. Moreover, resistance to TCS can also cause a cross-resistance to antibiotics [10]. McNamara et al. [11] and Carey et al. [8] have investigated how TCS impacts microbial communities in AD. In the first study mentioned, structural divergence in *Bacteria* and *Archaea* communities was observed when samples with TCS were compared to the control. These communities demonstrate methanogenic activity, which promotes the breakdown of organic substrates into CH_4. Moreover, Carey et al. [8] showed that relative abundance of *mexB*, a gene encoding a component of a multidrug efflux pump and a characteristic gene for TCS resistance, was significantly higher in samples with different TCS levels than in the control.

Table 1
Physicochemical characterization of the supernatants obtained at the end of feedings I, II, and III.

Feedings	TCS concentration (mg/L)	Parameters			
		pH	EC (mS/cm)	ORP (mV)	Soluble COD (mg/L)
I	7.8	7.5 ± 0.2	4.5 ± 1.3	-144 ± 15	904 ± 69
	15.7	7.7 ± 0.1	5.6 ± 0.1	-141 ± 7.4	1083 ± 245
	25.5	7.3 ± 0.1	4.4 ± 1.6	-147 ± 4.4	1280 ± 146
	31.4	7.8 ± 0.01	5.1 ± 0.3	-147 ± 1.9	1267 ± 277
II	7.8	7.7 ± 0.2	4.4 ± 0.6	-123 ± 10	1309 ± 95
	15.7	7.7 ± 0.2	5.0 ± 0.1	-157 ± 16	1108 ± 91
	25.5	7.6 ± 0.3	3.7 ± 0.4	-193 ± 16	1421 ± 61
	31.4	7.8 ± 0.2	4.1 ± 0.4	-150 ± 5.9	1939 ± 80
III	7.8	7.3 ± 0.2	5.2 ± 1.2	-187 ± 44	820 ± 169
	15.7	7.5 ± 0.2	6.1 ± 1.6	-190 ± 17	993 ± 293
	25.5	7.6 ± 0.3	4.9 ± 0.4	-164 ± 29	1107 ± 141
	31.4	7.8 ± 0.4	5.0 ± 0.1	-180 ± 23	1182 ± 146

Notes: I: first feeding, II: Second feeding; III: Third feeding; EC: electrical conductivity; ORP: oxidation–reduction potential; COD: chemical oxygen demand. All values are expressed as the mean ± standard deviation. $n = 3$.

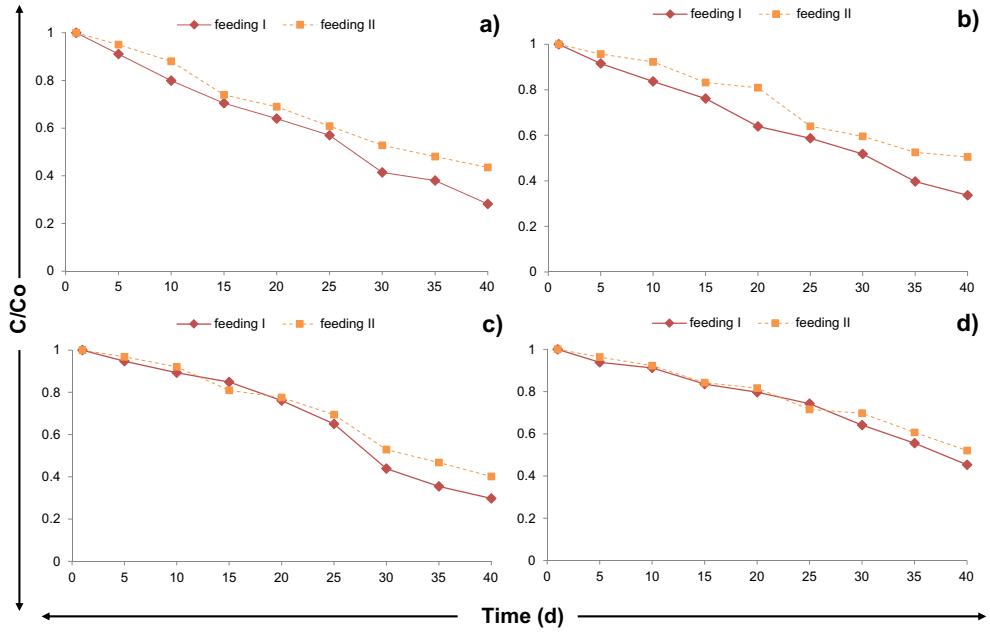

Fig. 4. Concentrations of TCS normalized by their influent concentration in the supernatant of each reactor evaluated during feedings I and II, considering the different TCS concentrations (mg/L) evaluated: (a) 7.8, (b) 15.7, (c) 23.5, and (d) 31.4.

3.3. TCS removal

Table 1 shows physicochemical characterization of the supernatants obtained at the end of feedings I, II, and III. Regarding pH values during the different feedings, the influence of TCS concentrations was not observed. At the beginning of the assays (0 d), the pH remained neutral (pH = 7), whereas at the end of the assays (40 d), pH values fluctuated between 7.3 and 7.8. Despite VFA accumulation, which represents an anomaly of the AD process, the pH ranges did not exhibit variations during this study. The optimum pH values of methanogenic bacteria fluctuated between pH 6.7 and 7.4, and when values were close to pH 6.2 and 7.8, the methanogenesis rates decreased [19]. The same tendency was detected for EC values, which varied between 3.7 and 6.1 mS/cm. Moreover, the ORP values fluctuated between -141 and -193 mV during the incubation period. These results showed that anaerobic conditions of reactors were maintained.

Fig. 4 shows the concentrations of TCS normalized by their influent concentration in the supernatant of each reactor evaluated in during feedings I and II considering the different concentrations of TCS evaluated. For all concentrations evaluated, the removal rates of TCS during feeding I were between 1.2 and 1.3 times higher than that during feeding II. Considering the initial concentrations of TCS, these values decreased between 55 and 71% and 47% and 61% during the feedings I and II, respectively. Regarding the effect of TCS concentration, the minimum removals were achieved for TCS concentration of 31.4 mg/L, with a decrease of 55% and 47% during feedings I and II, respectively. These results indicated that TCS was removed in the supernatant with values above 47%. However, because of their hydrophobic characteristics, TCS tends to be sorbed and accumulate in the biosolids [20]. Ying and Kookana [21] investigated the occurrence of TCS in effluents, biosolids, and surface water and its fate in WWTPs. In this study, the removal rates for TCS were found between 72 and 93%. However, adsorption onto the sludge played a significant role in the removal of TCS with a mean concentration of 5.6 mg/kg. Similarly, other studies reported a TCS removal rate of below 20% during the AD process. In this study, 90% of TCS was associated with biosolids, showing that TCS is a persistent compound under anaerobic conditions [9]. It is important to consider that the removal of micropollutants depends on several factors such as influent characteristics, physicochemical properties of the compound, and operational parameters of WWTPs (hydraulic retention time (HTR) and organic loading rates (OLR)) [22].

4. Conclusions

TCS exhibits an inhibition effect on methanogenic activity, which depends on the TCS concentrations. For TCS concentration of 7.8 mg/L, the maximum CH_4 production was achieved at values between 142.0 and 289.6 mg/L during different feedings. Despite the removal rates of TCS during AD above 42%, the methanogenic activity was not recovered during feeding III. This inhibition effect was corroborated by the VFA accumulation achieved during TCS concentrations of 23.5 and 31.4 mg/L.

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