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Methanogenic toxicity evaluation of chlortetracycline hydrochloride

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ABSTRACT

Background: Anaerobic digestion is a technology applied successfully to converting organic matter into biogas. However, the presence of inhibitory compounds such as antibiotics can adversely affect methane production. The aim of this study is to evaluate the toxic effect of chlortetracycline hydrochloride (CLOR) on the methanogenic bacteria. In order to study the methanogenic toxicity of CLOR, different concentrations of CLOR (10, 50, 100, 200 mg L^{-1}) were evaluated by methanogenic toxicity assays using three feedings.
Results: Maximum methane production was obtained for the assays with 10 mg CLOR L^{-1}, the values obtained were 277 ± 4.07; 193 ± 11.31 and 166 ± 7.07 mL for the first, second and third feedings, respectively. The average values for acetic, propionic and butyric acid at start of the experiments were 2104 ± 139; 632 ± 7.6; 544 ± 26 mg L^{-1}, respectively. The VFA values obtained finally of the experiment were dependent on the evaluated antibiotic concentrations, indicating that the efficiency of methanogenesis is directly affected by the CLOR concentration.
Conclusions: CLOR is an effective methanogenic bacteria inhibitor. Moreover, the results show that CLOR has a bactericidal effect on methanogenic activity given that methane production did not recover during the third feeding. This study shows that the 50% inhibitory concentration (IC_{50}) for methanogenic bacteria in 10 mg L^{-1}.

1. Introduction

Antibiotics are natural or synthetic chemical substance used extensively in human and animal medicine to treat diseases, prevent infection and promote growth [1,2]. In the environment antibiotics constitute a pollutant, which they enter mainly through discharges from wastewater treatment plants that are not designed to remove them [3,4]. Recent studies report the presence of these drugs in wastewater, groundwater and sewage sludge, with detected concentrations ranging from 0.1 to 100 μg L^{-1} [5,6,7,8].

Anaerobic digestion (AD) is a technology used to transform organic matter into methane. Organic substrates like sewage, manure, and agricultural wastes may contain antibiotics that inhibit methanogenic activity [9,10,11]. The stability and efficiency of AD processes depend on the microbial population, the biodegradability of the compounds and chemical characteristics [12]. The microorganisms involved in AD are sensitive to antibiotics that can reduce growth rates and disable the activity of microorganisms [10,13,14,15].

In previous works, Álvarez et al. [14] evaluated the effect of oxytetracycline and chlortetracycline hydrochloride (CLOR) during AD of swine manure. They observed that methane production was reduced by 56%, 60% and 62% at oxytetracycline and CLOR concentrations of 10, 50 and 100 L^{-1}, respectively. Arikan et al. [15] evaluated the effect of oxytetracycline in calf manure and found a 27% reduction in methane production at a concentration of 3.1 mg oxytetracycline L^{-1}. Furthermore, Sanz et al. [12] observed a reduction in methane production from 20 to 80% when the CLOR concentration increased from 2 to 150 mg L^{-1}.

CLOR is a broad-spectrum antibiotic used in human and animal medicine that acts by inhibiting bacterial protein synthesis. Table 1 shows the structure and physicochemical properties of CLOR. The main characteristics are high solubility (156.29 mg L^{-1}) and very low partition coefficient values (Log Kow: −3.60). Consequently, CLOR can inhibit bacteria activity. Sanz et al. [12] showed that CLOR is a powerful inhibitor of anaerobic bacteria, estimating that the 50% inhibitory concentration (IC_{50}) for methanogenic bacteria in 40 mg L^{-1}. They observed that CLOR concentration directly affects the activity of acetogenic and acetoclastic methanogenic bacteria. At concentrations above 200 mg CLOR L^{-1}, bacteria did not consume acetic acid and acetogenic bacteria used to consume butyric acid died at concentrations over 100 mg L^{-1}. They concluded that CLOR has selective effects on different microorganisms.

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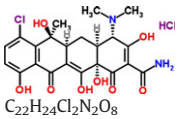
Table 1
Structure and physicochemical properties of CLOR.

Analyte (CAS number)	Structure	Molecular weight (g/moL)	Log K_{ow}	Log K_{oc}	Solubility (mg/L)
Chlortetracycline hydrochloride (64-72-2)	 $C_{22}H_{24}Cl_2N_2O_8$	515.15	−3.60	−1.56	156.29

Log K_{ow} y Log K_{oc} was obtained from EPI suite Program version 4.1.

The aim of the present study is to evaluate the toxic effect of CLOR on methanogenic bacteria.

2. Materials and methods

2.1. Analytes

Chlortetracycline hydrochloride (CLOR, 99%) was obtained from Sigma-Aldrich (Steinem, Germany). The volatile fatty acids (VFA; acetic acid, propionic acid and butyric acid), $CaCl_2 \times 2H_2O$, NaOH, $MgSO_4 \times 7H_2O$, $Na_2S \times 9H_2O$, K_2HPO_4 were obtained from Merck (Darmstadt, Germany). $NaHCO_3$ and NH_4Cl were purchased from Winkler (Santiago, Chile).

2.2. Inoculum

The anaerobic biomass used in the methanogenic toxicity assay was obtained from an anaerobic treatment system of a brewery. This biomass is a granular sludge type. The sludge in the study presented the following characteristics: pH 7.13, volatile suspended solid (VSS) 30.88 mg L^{-1} and total suspended solids (TSS) 48.57 mg L^{-1}. The initial methanogenic activity of the sludge was 0.31 g COD_{CH_4} $gVSS^{-1}$ d^{-1} (COD: chemical oxygen demand).

2.3. Methanogenic toxicity assays

The methanogenic toxicity assays were carried out in 100 mL of total volume (the glass serum bottle volume was 125 mL) using a VFA mixture as substrate and CLOR as the toxic to evaluate, following the methodology previously described [16,17].

Methane production was measured by displacement of an alkaline solution of NaOH 2.5%. The final concentration of each VFA in the reactor (bottle) was: acetic acid 2 g L^{-1}, propionic acid 0.5 g L^{-1} and n-butyric acid 0.5 g L^{-1} (total COD from VFA was 3.8 g COD-VFA L^{-1}). The VFA solution was previously neutralized (pH: 7) with NaOH. The media also contained the following nutrients: NH_4Cl (0.14 g L^{-1}), K_2HPO_4 (0.125 g L^{-1}), $MgSO_4 \times 7H_2O$ (0.10 g L^{-1}), $CaCl_2 \times 2H_2O$ (0.01 g L^{-1}) and $NaHCO_3$ (0.2 g L^{-1}). The inoculum concentration added to each reactor was 1.77 g SSV L^{-1}. The anaerobic conditions were secured by adding 100 mg $Na_2S \times 9H_2O$ L^{-1}. Each reactor was sealed and bubbled with nitrogen gas for 2 min in order to remove air from the headspace. Finally, samples were incubated at 35°C throughout the experiment.

Three successive feedings to each antibiotic concentration were carried out. In the first feeding, the sludge was exposed to media containing CLOR and VFA substrate. At the end of the first feeding, the spent medium (liquid phase) was carefully decanted and the sludge was again exposed to CLOR and VFA substrate. At the end of the second feeding, the spent medium was removed. In order to evaluate residual sludge activity after the first and second exposure, a third feeding containing only the VFA mixture solution as substrate was added to culture bottles. The assays were carried out at 37°C and incubated for 18 d.

The liquid fraction (supernatant) obtained for each reactor after the feeding was stored and subsequently analyzed (pH, conductivity, redox potential and COD).

The CLOR concentrations evaluated were: 0 (control), 10, 50, 100 and 200 mg L^{-1}. All assays were conducted in triplicate.

2.4. Analytical methodology

Physicochemical parameters: conductivity, redox potential, and pH were measured using a multiparametric OAKTON-PC650 (Eutech Instruments, Singapore).

Water quality parameters: COD, SST and VSS were determined according to the methodologies established in Standard Methods, specifically through the following procedures: the 5220-C method for COD; the 2540-D method for TSS and the 2540-E method for VSS [18].

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, United States). The carrier gas was nitrogen (purity 99.999%) at a constant flow of 2.23 mL min^{-1}. The oven temperature was held at 95°C for 1 min, then temperature programmed at 10°C min^{-1} 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).

3. Results and discussion

3.1. Effluent characteristics

Table 2 shows the average values and standard deviation (N = 3) obtained for the physicochemical parameters (pH, conductivity, redox potential and total COD) from each assay, in function of the evaluated antibiotic concentration.

The presence of CLOR in the reactor did not affect pH during the digestion process. The pH level at the start of the assay was approximately 7 for the blank and each CLOR concentration evaluated, while by the end of the digestion of period (D 18) pH had increased slightly, with values ranging from 7.3 to 8.1. Several authors have reported that the optimum pH range for methanogenic bacteria is between 6.7 and 7.4, while the methanogenesis rate decreases at pH values < 6.2 or > 7.8 [19,20,21]. No negative effects on the microorganisms responsible for AD were observed as a result of any change in pH.

Specific conductivity values ranged from 2.83 to 9.14 mS cm^{-1}. In the absence of CLOR or at low concentrations of the antibiotic (10 and 50 mg CLOR L^{-1}) low conductivity values were observed, while high CLOR concentrations (100–200 mg CLOR L^{-1}) were associated with high conductivity values of conductivity, suggesting a relationship between CLOR concentration and conductivity.