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Research Article

Bioavailability and dosing strategies of mineral in anaerobic mono-digestion of maize straw

The influence of the bonding form distribution of Fe, Ni, Co and Mn and their potential bioavailability during the anaerobic degradation of maize straw was investigated. Two reactors were operated over 117 days at 37°C and different dosage strategies of mineral were studied in reactor (R2). Control reactor (R1) was metal-limited over time. mineral supplementation (1 g L^{-1}) once a week reported the highest methane yield (257 mL g^{-1} VS) with 30% of increment. Ni and Co predominated in their oxidizable bonding forms and Fe mainly existed as residual and oxidizable fractions. The potential bioavailability (Mn >> Co ≈ Ni > Fe) of R2 was higher comparing to R1. Metal deprivation in R1 led to depletion of both sequential extraction fractions and total metal concentrations until the end of the process. This study confirmed that the dosage strategy of mineral has a stimulatory effect on methane production from crop maize waste.

Keywords: Anaerobic degradation / Methane yield / Mineral supplementation / Potential bioavailability / Speciation

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1 Introduction

The use of energy crops alone as the feedstock for biogas production has been previously studied [1–5]. However, anaerobic digestion (AD) of these biomass has mainly been limited by its slow hydrolysis [4], which is attributed to the complex and rigid matrix of plant cells; that it is resistant to enzymatic attack because of the tight association between lignin, cellulose, and hemicellulose [6]. Another drawback of the AD of lignocellulosic materials is its poor content of nutrients, primarily micronutrients such as Fe, Ni, Co, Mo, Mn, S and W, which are involved in the synthesis of essential coenzymes or cofactors in methanogenic pathways [7]. Therefore, its supplement is a prerequisite for microbial growth and metabolism in AD. Studies on AD of straw have demonstrated that both hydrolysis and nutrients deficiency were the main causes of poor process-performance [8].

Different pre-treatment studies have been addressed toward an improvement of the anaerobic biodegradability of crop residues [2,6,9]. Others research on trace elements supplementation in mono-digestion of straw and silage with the goal to enhance the performance and stability of the process [2,3,5]. From

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Abbreviations: BMP, biochemical methane potential; **HRT**, hydraulic retention time; **OLR**, organic loading rate; **SCSTR**, semicontinuous stirred tank reactors; **SE**, sequential extraction; **TS**, total solids; **VFA**, volatile fatty acid; **VFA/TIC**, volatile fatty acids/total inorganic carbon ratio; **VS**, volatile solids the mentioned studies, a clear stimulating effect on methane yield has been reported to be ranging from 350 to 429 mL CH_4 g^{-1} VS, which were 15 to 36% higher than that of the substrates without trace metal supply, when using Fe-Ni-Co-Mo, individually or in combination. The previous data resulted from addition of trace metals in form of salts. However, in recent studies, the trace metals supply from natural mineral has demonstrated to have a similar effect during the biodegradation of microcrystalline cellulose (synthetic model substrate) and maize straw in batch and semi-continuous experiments, respectively [10, 11]. From their findings, the highest methane yield of 397 and 257 mL CH_4 g^{-1}VS, were obtained in the AD of microcrystalline cellulose and maize straw, respectively, with mineral addition, which was 31% higher than that the substrates without mineral supply. For it finds of practical importance, the use of the natural mineral, is an alternative to other micronutrient source widely applied.

In Cuba, there are mineral clays with a varied composition of nutrients that can be useful to the AD. Those minerals have metals (e.g., Fe, Ni, Co, Mn) content, which can be promoters of the AD. Nevertheless, so far no studies have been found addressing the stimulatory effect of natural mineral and metals speciation/bioavailability to enhance the anaerobic digestion of maize straw in semi-continuous reactors. Therefore, there is a clear need for concerted efforts to utilize state-of-the-art information on chemical speciation and nutritious metals to improve our understanding of their bioavailability in the reactors, and to clarify the stimulatory effect on the process.

The present study is focused on the chemical speciation of Fe, Ni, Co and Mn, and bioavailability during the operation of semi-continuous stirred tank reactors (CSTRs) with natural mineral supplementation. In addition, the consequent effects on their bio-uptake processes were assessed.

2 Materials and methods

2.1 Feedstock, inoculum and mineral

Maize straw collected from an agricultural farm in Campinas (São Paulo) was used as feedstock for the anaerobic digestion. In order to assist the daily input of feedstock the particle size of the biomass was reduced to 0.5 mm and stored at 4°C until used.

Inoculum was collected from an up-flow anaerobic sludge bed (UASB) reactor treating poultry slaughterhouse wastewater (DACAR, Tietê-SP, Brazil) and was prepared following the guidelines for the fermentation of organic matter [12]. Prior to use, the inoculum was smoothly liquefied and then placed in fermenters. Maize straw was fed at a moderate organic loading rate (OLR) of 0.5 g VS L^{-1} d^{-1} operating under mesophilic condition of 37 ± 1°C. The feeding process was stopped after 4 weeks of adaptation. The biodegradation process continued along two weeks in order to remove the residual degradable components. The inoculum was filtered to eliminate particle size larger than 3 mm and the filtrate was used to inoculate the reactors.

A natural clay mineral mixture with a particle size from 0.12 to 0.25 mm was provided by the Cuban Research Centre for the mining and Metallurgical Industry (CIPIMM). Table 1 presents the characteristics of feedstock, inoculum and mineral used in this study.

Table 1. Composition of inoculum, feedstock and mineral used in the semi-continuous fermentation tests

Parameters	Inoculum[a)]	Maize straw	Mineral
TS (% w/w)	4.5	90.2	n.a[b)]
VS (% TS)	69.0	95.9	
Fiber (%TS)			
Cellulose	n.a[b)]	36.2	n.a[b)]
Hemicellulose		48.3	
Lignin		17.9	
Metal (mg g^{-1} TS)			
Fe	17.98	0.11	296.17
Ni	0.06	0.01	3.56
Co	0.01	0.001	0.58
Mn	0.21	0.06	4.88
Al	-	-	24.71
Cu	-	-	0.04
Ca	-	-	39.79
Mg	-	-	71.66

a) The inoculum concentrations are the average of duplicate samples.
b) Not analyzed.

2.2 Biomass sampling

Sampling took place on start-up of the reactors SCSTRs and after 57, 73 and 96 days of operation. The reactors were previously stirring during 10 min before of the biomass sampling. Effluent was taken from the surface of the reactors with a syringe. The liquid and solid phases were separated by centrifugation for 10 min at 10 000 rpm and the supernatant was filtered through a microfiber filter (0.45 μm). Both phases were stored in polyethylene tubes at 4°C.

2.3 Experimental set-up

The experiments were performed in two 1-L (0.8 L effective volume) CSTRs as described by González-Suárez et al. [11] for a period of 117 days of investigation. The reactors were operated under mesophilic conditions at a temperature of 37 ± 1°C. A hydraulic retention time (HRT) of 30 days and OLR of 0.5 g VS $L^{-1}d^{-1}$ to 2.0 gVS $L^{-1}d^{-1}$ were applied to the reactors.

R1 was operated without mineral supply (deprived- reactor), whereas the reactor R2 was supplemented with 1 g L^{-1} mineral in the influent from day 27 till the end of the experiment (day 117) at different dosage periods defined as: I (once a day), II (without mineral supply), III (once a week) and IV (twice a week). This mineral dose was selected in previous studies carried out during the batch anaerobic digestion with the same substrate (maize straw) at different mineral doses: 10, 50 and 1000 mg L^{-1} (data not shown). From these results, the highest methane yield of 244.9 mL CH_4 g^{-1}VS was obtained with 1 g L^{-1} of mineral, which was up to 31.6% higher than untreated straw.

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Feeding was carried out once a day with amounts of the feedstock (according to the applied OLR) and mineral previously defined. Volumetric biogas production was continuously measured online with a Ritter Milligas Counter (Ritter, Germany) and methane content of biogas was determined using a gas chromatographic analysis (Shimadzu GC-2010) as described González-Suárez et al. [11]. The maximum expected methane yield from maize straw was obtained by a modified biochemical methane potential (BMP) test (data not shown) according to Pagés et al. [13].

2.4 Total metal concentration

The total metal (Fe, Ni, Co and Mn) concentration of approximately 1 g wet sludge per g of total solid (TS) was determined after aqua regia destruction. After acid digestion, the samples were filtered (0.45 μm) and diluted to 0.1 L using ultrapure water (Milli-Q System-UPW). The total metal concentration was measured using atomic absorption spectrometry (AAS, 240FS Varian Atomic Absorption Spectrometer).

UPW was used in preparing stock solutions of all reagents. All chemicals and solvents used were of analytical grade and obtained from Sigma Chemical Company. Glassware was treated in a 7 M HNO_3 acid bath at least 12 h prior to use.

2.5 Sequential extraction procedure

The bonding forms were determined with a modified Tessier sequential extraction (SE) procedure as described by Osuna et al. [14] (Table 2). This methodology has a relatively good precision and application in several studies on metal speciation in anaerobic reactors [15,16]. The defined fractions for the SE were sludge liquid phase, referred as: exchangeable (i), carbonates (ii), oxidizable (iii) and residual (iv). Reducible fractions, e.g. metals bound to Fe oxides, are not extracted because their extraction tends to oxidize the anoxic samples and thus overestimates metals bound to oxidized compared to reduced compounds [16]. The extractions were performed in triplicate on sub-sample of about 1 g wet sludge (based on TS). Through each extraction step, the sub-samples were centrifuged and finally filtered as described above (2.2). Metals content of the liquid phase after SE-step was also analysed by AAS. The sludge was rinsed with 25 mL of UPW, centrifuging and discarding the supernatant. The reagent for extraction corresponding to the next fraction was added to the tubes. This procedure was applied in each SE-step. The residual fraction was digested with *aqua regia* as performed with the total metal extraction method.

The recovery rate of each element analyzed during the SE procedure was determined as the sum of the metals content extracted after each SE-step related to the total metal concentration in the fresh sample after acid digestion in non-fractionated sludge sample.

According to Tessier et al. [17], the bonding form distribution can be used to assess the potential bioavailability of trace metals. These fractions possess a decreasing solubility/reactivity from the first fraction (the most bioavailable) to the last fraction (the least bioavailable), which allow to evaluate the potential bioavailability of metals in anaerobic reactors.

Table 2. Modified Tessier sequential extraction procedure

Fraction	Extracting agent	Extraction conditions	
		Shaking time[a]	Temperature (°C)
Exchangeable	10 mL NH_4CH_3COO (1M, pH=7)	1 h	20
Carbonates	10 mL CH_3COOH (1M, pH=5.5)	1 h	20
Organic matter/sulfides	5 mL H_2O_2 (30%, pH=2)	3 h	35
Residual	10 mL *Aqua regia* (HCl/HNO_3, 3:1)	30 min	Microwave digestion 120°C

[a] Shaking was applied at 100 rpm.

2.6 Statistical analyses

The statistical data analyses were performed using STATGRAPHICS Centurion XV.II as statistical software package. One-way ANOVA and Tukey's multi-comparison test were conducted to assess the effects of natural mineral supply on the methane yield during semi-continuous mono-digestion of maize straw. The mean Y_{CH4} at different dosing phases within and between both reactors were also compared and the standard deviations (SD) were pooled for each studied phase. Statistical significance was calculated for the metal content of each reactor evaluated.

3 Results and discussion

3.1 Effect of mineral dosage strategies on semi-continuous anaerobic digestion

The effect of different mineral dosing strategies on the process performance was evaluated in terms of methane yields. Figure 1 shows the evolution of methane yield achieved in the SCSTRs and it is compared to the one reported by BMP experiments. In addition, the volatile fatty acids (VFA) to total inorganic carbon (TIC) ratio and pH remained almost constant over the whole experiment (Fig. 2). The pH for the reactors R1 and R2 was around the neutral (7.06–7.80).

Despite mineral application once a day (Phase I) in R2 only slight differences (not statistically significant) in methane yields were observed between both reactors until day 60 (Phase I and II). During this period, the VFAs to alkalinity ratios for both reactors were <0.2 (Fig. 2). It has been reported that instability AD processes are likely to occur at VFAs to alkalinity ratios ≥ 0.5 [7]. However, the amount of methane in the biogas in R1 and R2 decreased in 8% and 12%, respectively, compared to Phase I.

In order to reach higher methane yields and to enhance the stability of the anaerobic digestion in R2, the dosage strategy

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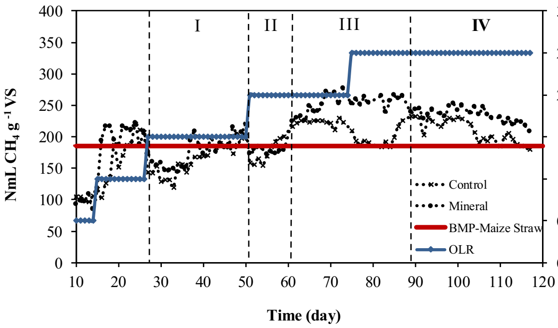

Figure 1. Specific methane yields in mono-digestion of maize straw with and without mineral supply. Dashed lines represent the changes in dosing strategy in R2.

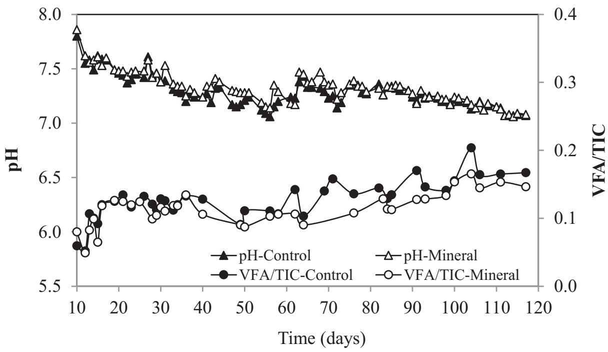

Figure 2. Variation in pH and VFA/TIC ratio in mono-digestion of maize straw with and without mineral supply.

of mineral was changed from once a day to once a week at day 61 (Phase III). Before starting this strategy, a period of 10 days without mineral application was applied in R2 (Phase II) and the OLR was increased to 2.0 g VS L^{-1} d^{-1}. In this period both reactors showed process stability in terms of pH and VFA/TIC ratio (Fig. 2).

On day 61 (Phase III), mineral was supplied once a week during 28 days (Fig. 1). Furthermore, the OLR was increased up to 2.5 g VS L^{-1} d^{-1} at day 75. In this third phase, differences in process performance became more obvious. The applied dosage strategy to R2 exhibited the maximum value of methane yield (257 ± 8 mL CH_4 g^{-1}VS), with a significant increase by 30% compared to R1 (198 ± 14 mL CH_4 g^{-1}VS). Previous studies have reported the effects of trace elements supplementation in terms of methane yield during mono-digestion of maize straw or maize crop. For instance, Liu et al. [18] reported that the combination of Fe, Ni and Co in 1.0, 0.4, and 0.4 mg L^{-1}, respectively, attained methane yield of 243 mL CH_4 g^{-1} VS, which was 62% higher than with untreated corn stover. Likewise, Khatri et al. [2] performed Fe dosing from range 50 to 2000 mg L^{-1} with maize straw and obtained the highest methane yield of 350.2 mL CH_4 g^{-1} VS at 1000 mg L^{-1}, with a 16.5% increase respect to straw untreated. This high methane yield could be due to the lower content of lignin (3.82% TS) in the maize straw and thus easier microbial degradation of the holocellulose compared to content of lignin of the straw used in this study (17.9% TS). The obtained results using minerals are comparable to the inorganic salts addition, which is profitable from the practical point of view as technological alternative.

It is remarkable to notice that during this period, the reactor R2 was able to overcome the OLR increment due to the strategy of mineral supplementation. Hence, it can be concluded that mineral supplementation once a week at 1g L^{-1}, provides the required nutrients for mono-digestion of maize straw and stimulated methane production.

In contrast, during Phase IV methane yield decreased in 11% and 9% in comparison to the methane yield in Phase III, for R1 and R2 respectively, with statistically significant differences. R1 (metal-limited) was not able to keep a proper performance at 2.5 g VS L^{-1} d^{-1}. In the case of R2, the increment in mineral dosage from one to two-fold in combination with the applied OLR, led to diminish the substrate degradation efficiency, with a tendency to decrease the methane yield until the end of the experiment in both reactors. These results are in agreement with Zhang et al. [7], who demonstrated the excessive addition of Fe (1000 mg L^{-1}) and Ni (50 mg L^{-1}) exhibited the obvious toxicity to methanogens with the reduction of methane yield.

In general, methane yields achieved during Phase III and IV in R2 were on average higher and significantly different from the respective BMP-maize straw yields (186 mL CH_4 g^{-1} VS) and R1-SCSTR. It is interesting that R2 had a methane content of 50% approximately while R1 showed methane content values lower than 47%. Though the metals trace source and dosage strategy obtained in this study were only in partial agreement with previous researches [5,7] due to the characteristics of mineral, substrate, inoculum and different operating conditions, the results confirmed the simulating effect of mineral supplementation once a week on methane yield from maize straw.

Since the bioavailability of nutrients is not always related to the total amount [5] but to the metal concentration supplied, it is necessary to discuss the bioavailability, retention, accumulation and release of natural mineral during the anaerobic digestion of maize straw.

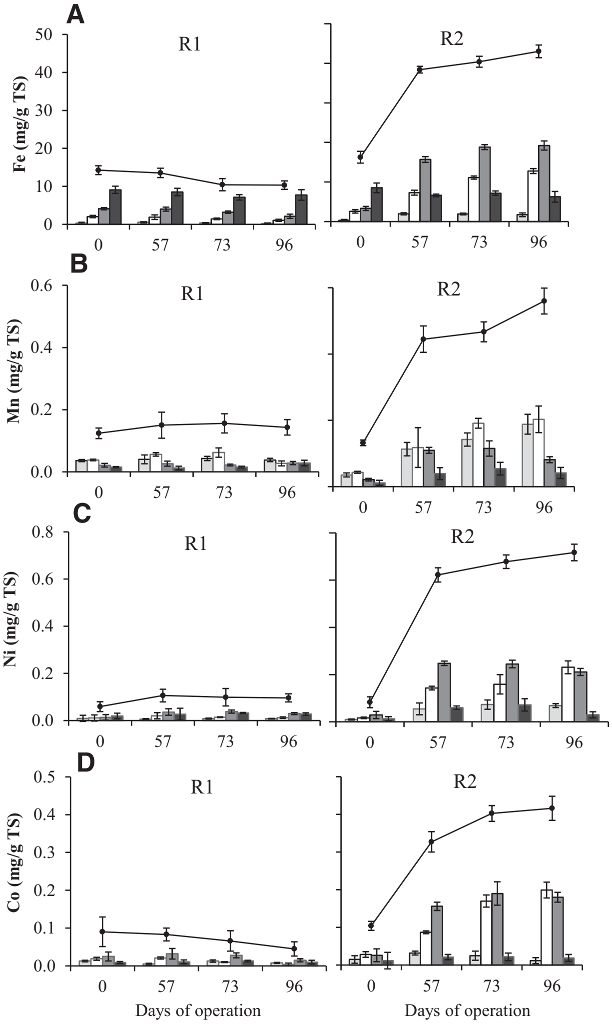

Figure 3. Bonding form distribution and total concentrations of (A) Fe, (B) Mn, (C) Ni and (D) Co from control reactor- R1 and mineral supply- R2. ▨ Exchangeable; □ Carbonates; ▩ Oxidizable; ■ Residual; Total metal. Standard deviations are given as error bars.

3.2 Effect of mineral, trace metals speciation, and bioavailability during anaerobic digestion

The understanding of the chemical speciation of metals and its bioavailability is essential for a proper dosage strategy of metal addition in anaerobic digestion [15]. In order to assess the effects of mineral supply (Fe, Ni, Co, Mn) and their bioavailability during the mono-digestion of maize straw, the bonding form distribution of these metals at the starting-up (day 0), 57, 73, and 96 days of operation were determined for both reactors (Fig. 3). The chemical speciation of these four metals showed different changes in the reactors with and without mineral supply during the mono-digestion of maize straw. Concentrations of Fe, Ni, Co and Mn in the solid phase, calculated by the sum of the SE-fractions, were mostly in the same range as the concentrations measured with the total acid digestion method (Table 3). Recovery from the sum of the SE-fractions in function of the total acid digestion was included between SD of ± 20% except for Ni (73 and 75%) and Co (73 and 78%) for R1 and R2, respectively, assuring the validity of the results (Table 3). The values are similar to those recorded in the literature for the same sequential extraction scheme [15,16]. The amount of metals extracted during the total acid digestion in some case was higher than the amount extracted during the SE-procedure. This difference could be due to a loss of material during the different stages of the procedures, e.g. extractions, centrifugation or washing.

In general, the results of Fe, Ni, Co and Mn from SE for the reactor without mineral supply (R1) indicate a low bioavailability of these metals (Table 3) with a trend to decrease over time (Fig. 3). As it can be noticed, Fe was mainly associated to the residual fraction (61%), considered unavailable for cell uptake, and followed by oxidizable (25%) fraction (Fig. 3A).

Fuentes et al. [19] analyzed anaerobically digested sludge and found that most of the iron was extracted in the residual fraction (90%). In reactor R1, the exchangeable (3%) and carbonate (12%) fractions of Fe remained almost negligible with regard to both residual and oxidizable fractions (Table 3). The bonding forms of Ni and Co mainly existed as oxidizable fractions (36 and 42%, respectively), with a trend to diminish over time (Fig. 3C and D). This behavior is expected given the affinity of organic matter for both metals and the formation of stable complexes [19], although a significant part of Ni (34%) was extracted from the residual fraction, implying a low potential bioavailability of this metal. On the other hand, Mn showed the higher degree of bioavailability due to the high proportion of metal mobilized in the exchangeable (31%) and carbonate (36%) fractions (Fig. 3B). These fractions are considered to contribute to bioavailability for metals uptake by microorganisms [16,19]. However, it must be borne in mind that the total quantity of Mn during the experiment was low. Callander and Barford [20] determined that manganese precipitated as manganese carbonate in anaerobic digesters, except in the case of high sulphide concentration.

At the end of experiment (day 96) the more bioavailable fractions of Fe, Ni, Co and Mn significantly decreased by 23, 32, 33, and 20%, respectively, compared to the initial phase in R1 (day 0) (Fig. 3 and Table 3). The results confirm that fewer metals were directly bioavailable for microbial uptake at the end of the process, which had a negative impact on both archaea and bacteria community structure under mineral-limited conditions [11], and hence on the methane yield. A decrease by 11% of methane yield in this reactor, was observed when the OLR was increased to 2.5 g VS L^{-1} d^{-1} on day 75 (Fig. 1). As proved by Pobeheim et al. [5], limitations of Ni and Co led to the decrease of biogas production until 25 and 10%, respectively, during mesophilic anaerobic digestion of maize silage in semi-continuous reactors. Similarly, previous investigation indicated a negative effect of Ni/Co limitations on anaerobic digestion [15,21].

This suggests that metals content in the inoculum were present in enough concentration to support the methane production stable during the start-up in this reactor, but not in enough quantities to maintain trace element requirement during a long time period of digestion, leading to a lower microbial activity and hence instability in the control reactor.

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Table 3. Metal concentrations [a] for R1 and R2 in the SE-fractions performed at 0, 57, 73, and 96 days of operation

Day	Fraction	R1 (mg/g TS)				R2 (mg/g TS)			
		Fe	Ni	Co	Mn	Fe	Ni	Co	Mn
0	Exchangeable	0.32 ± 0.23	0.009 ± 0.013	0.013 ± 0.002	0.04 ± 0.003	0.31 ± 0.23	0.01 ± 0.002	0.02 ± 0.010	0.04 ± 0.006
	Carbonates	2.04 ± 0.31	0.011 ± 0.013	0.019 ± 0.004	0.04 ± 0.003	2.58 ± 0.44	0.02 ± 0.003	0.03 ± 0.008	0.04 ± 0.003
	Oxidizable	4.12 ± 0.26	0.013 ± 0.012	0.025 ± 0.012	0.02 ± 0.006	3.31 ± 0.52	0.03 ± 0.015	0.03 ± 0.017	0.02 ± 0.004
	Residual	9.08 ± 0.95	0.019 ± 0.012	0.009 ± 0.003	0.02 ± 0.002	8.52 ± 1.23	0.01 ± 0.009	0.01 ± 0.022	0.01 ± 0.008
	ΣSE[b]	15.56	0.052	0.066	0.11	14.72	0.07	0.08	0.11
	Recovery (%)	109	88	73	89	90	83	78	83
57	Exchangeable	0.55 ± 0.15	0.006 ± 0.002	0.005 ± 0.002	0.04 ± 0.014	1.97 ± 0.27	0.05 ± 0.025	0.03 ± 0.005	0.11± 0.020
	Carbonates	1.89 ± 0.62	0.020 ± 0.013	0.021 ± 0.003	0.06 ± 0.006	7.28 ± 0.62	0.14 ± 0.008	0.09 ± 0.004	0.12 ± 0.060
	Oxidizable	4.01 ± 0.53	0.036 ± 0.014	0.042 ± 0.014	0.03 ± 0.008	15.69 ± 0.74	0.26 ± 0.009	0.15 ± 0.011	0.11 ± 0.009
	Residual	8.53 ± 0.97	0.027 ± 0.025	0.011 ± 0.005	0.01 ± 0.006	6.62 ± 0.33	0.06 ± 0.007	0.02 ± 0.007	0.04 ± 0.018
	ΣSE	14.98	0.089	0.069	0.13	31.56	0.51	0.29	0.38
	Recovery (%)	111	84	83	89	82	81	91	85
73	Exchangeable	0.34 ± 0.10	0.008 ± 0.002	0.013 ± 0.003	0.04 ± 0.007	1.89 ± 0.22	0.07 ± 0.020	0.02 ± 0.012	0.14 ± 0.020
	Carbonates	1.45 ± 0.21	0.014 ± 0.001	0.010 ± 0.001	0.06 ± 0.015	11.13 ± 0.41	0.16 ± 0.041	0.17 ± 0.016	0.19 ± 0.015
	Oxidizable	3.21 ± 0.32	0.038 ± 0.007	0.028 ± 0.007	0.02 ±0.003	18.81 ± 0.61	0.25 ± 0.016	0.19 ± 0.031	0.12 ± 0.023
	Residual	7.11 ± 0.71	0.032 ± 0.002	0.013 ± 0.002	0.02 ± 0.003	7.18 ± 0.56	0.07 ± 0.026	0.02 ± 0.010	0.05 ± 0.019
	ΣSE	12.11	0.092	0.064	0.14	39.01	0.55	0.41	0.50
	Recovery (%)	116	93	97	91	97	81	101	107
96	Exchangeable	0.23 ± 0.11	0.008 ± 0.001	0.008 ± 0.001	0.04 ± 0.005	1.72 ± 0.47	0.07 ± 0.008	0.01 ± 0.008	0.19 ± 0.030
	Carbonates	1.06 ± 0.29	0.012 ± 0.003	0.004 ± 0.003	0.03 ± 0.008	12.75 ± 0.62	0.20 ± 0.027	0.19 ± 0.021	0.20 ± 0.040
	Oxidizable	2.12 ± 0.54	0.030 ± 0.004	0.015 ± 0.004	0.03 ± 0.005	19.22 ± 1.15	0.30 ± 0.015	0.18 ± 0.013	0.08 ± 0.009
	Residual	7.73 ± 1.41	0.028 ± 0.005	0.010 ± 0.005	0.03 ± 0.009	6.25 ± 1.36	0.03 ± 0.013	0.02 ± 0.009	0.04 ± 0.017
	Sum	11.14	0.077	0.037	0.12	39.94	0.54	0.41	0.51
	Recovery (%)	108	80	82	85	93	75	99	92

[a] Mean value ± standard deviation ($n = 3$).
[b] Sum of the elements extracted after each SE step.

On the contrary, with the mineral supplementation in R2 the total concentrations of Fe, Ni, Co and Mn showed a noteworthy increase compared to the initial state (Fig. 3, Table 3). During anaerobic digestion, the dominant speciation of Fe, Ni and Co was the oxidizable fraction. Gonzalez-Gil et al. [22], reported that sulphides forms are bioavailable for methanogenic consortium in anaerobic digestion and in particular, formed Co- and Ni- sulphide precipitates may serve as a micronutrient source over time [21]. Gustavsson et al. [15] observed a stimulatory effect of Ni and Co, despite its association to the oxidizable fraction. Likewise, Zhang et al. [7], investigated the changes of metals speciation during fermentation of food waste. From their findings, the Co (39.1%) and Mo (62.9%) elements mainly existed as oxidizable fractions.

With the dosage strategy of mineral once a week (Phase III) in R2, a significant amount of Fe, Ni, Co and Mn was found in the bioavailable fractions (exchangeable and carbonate) after 57 days of operation (29, 39, 40, and 61%, respectively), with exception of the residual fraction that only showed slight changes as shown in Fig. 3. This last one fixed in the crystal lattice of the mineral components is the least bioavailable fraction of metals [23]. On day 73, the values were increased to 33, 42, 48, and 66%, respectively. This rise did not change the pattern of the speciation but there was an amplification of the dominance of the first fractions, leading to an increment of all four-trace metals as adsorbed or easily extractable precipitates.

During Phase III, a remarkable stimulation of methane yield was observed (Fig. 1); reaching maximum methane yield value (257 ± 8 mL CH_4 g^{-1}VS). As previously published by González-Suárez et al. [11], this result is supported by a microbial dynamic evaluation with significant shifts in both bacteria and methanogens communities in comparison to the deprived reactor. These authors concluded that the dominant archaea populations were influenced by mineral addition, suggesting that methanogens had a stronger response to the concentration of supplemented mineral according to the strategy applied in this phase. In relation to the results of the present study, the stimulatory effect of these trace metals promoted the metabolic activity during mineral supplementation once a week in R2.

As known, Fe, Ni, Co and Mn are required in the synthesis of coenzymes and cofactors, which explains the high dependence of methanogenic activity on bioavailability of these metals.

On day 96, with the increase of mineral dosage by two-fold (Phase IV) in R2, the more bioavailable fractions of Fe, Ni, Co and Mn were 1.1, 1.3, 1.1, 1.2-fold of that in the reactor at 57 day of operation. However, the excessive supplementation of mineral in R2 caused the reduction of methane yield by 9%. The results confirmed the findings of Fermoso et al. [24], who suggested that the effects of trace metals were highly depended on the supplemental concentrations.

The potential bioavailability of metals can be assessed from the bonding form distribution, taking into account that the

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metal-binding forms with high solubility have a higher potential to be available for microbial uptake. The fractions possess a decreasing solubility/reactivity from the first to the last step and this decreasing solubility can be used as a measure of potential bioavailability [16], regarding the first two fractions, i.e. exchangeable and carbonates, the most bioavailable and the residual fraction is the least bioavailable. It was determined that during the operation time of the bioreactors, the ranking of potential bioavailability was the same in each reactor and both reactors had the highest Mn-bioavailability. Potential bioavailability was different for each metal in R1 (Mn >> Co > Ni > Fe), while in R2 the bioavailability of Ni and Co was similar (Mn >> Co ≈ Ni > Fe). The potential bioavailability of R2 was higher in comparison to R1. Thereby, the high metals availability was able to support the metals uptake by methanogens. The results obtained in this study confirmed that the dosage strategy of mineral once a week applied in R2 had a positive effect on methane production from maize straw, even when a significant part of Fe, Ni and Co was partially associated to oxidizable fraction.

4 Concluding remarks

The mineral clay is a potential source of micronutrients and it effect on methane yield is highly depended on applied dosage strategy.

Trace elements limitation in the maize straw fed CSTR reactor during a long time operation can induce instability and failure of process.

The best mineral dosage strategy for maize straw mono-digestion was once a week at 1 g L^{-1}, which exhibited remarkable stimulative effects on methane yield compared to the reactor without mineral supplementation.

A significant amount of Mn was bioavailable for microbial uptake, while the oxidizable fraction of Fe, Ni and Co dominated the bonding form distribution in anaerobic sludge and had therefore a stimulatory effect on the methane yield in the reactor with mineral supplemented.

Practical application

The anaerobic mono-digestion of lignocellulosic materials is frequently depressed at long time of operation. Metal supplementation is needed to avoid the diminishing of methane yield and to recover the overall process. Therefore, the regular application of metal cocktail of trace elements, in chemical salts, is a common practice to overcome these problems. On the other hand, minerals have metal content which can be released to the anaerobic medium in order to provide the requirements of micronutrients. The substitution of chemical salts by minerals is barely studied. Hence, the understanding of the bonding form distribution and bioavailability of metals in the anaerobic digestion is important to optimize the mineral dosage strategies to reach a stimulatory effect on methane production.

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