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Research Article

Increasing the loading rate of continuous stirred tank reactor for coffee husk and pulp: Effect of trace elements supplement

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In this study, the anaerobic performance and stability of coffee husk and pulp with and without trace element (TE) supplement was investigated, using 20 L mesophilic continuous stirred tank reactors for 140 days of experiment (DOE). The TE was cocktail of trace metals composed of Fe, Ni, Zn, Co, Mn, Mo, Se W and B. The organic loading rate (OLR) was increased stepwise from 2.5 (HRT = 40 d) to 6.0 kg VS m⁻³ d⁻¹ (HRT = 16.7 d). The highest methane productivity from pulp with and without TE was 1.272 and 0.965 m³ m⁻³ d⁻¹ at an OLR of 6.0 and 5.0 kg VS m⁻³ d⁻¹; while the husks performed 0.895 and 0.795 m³ m⁻³ d⁻¹ respectively, both at an OLR of 6.0 kg VS m⁻³ d⁻¹. The specific methane yield (SMY) of pulp (at OLR = 5 kg VS m⁻³ d⁻¹) with and without TEs was 217.9 ± 4.7 and 193.1 ± 8.2 L kg⁻¹ VS; while husk yielded 149.2 ± 6.0 and 132.5 ± 4.9 L kg⁻¹ VS, respectively. The effect of TEs on SMY was statistically significant (*p* < 0.01) at higher OLRs (5.0–6.0 kg VS m⁻³ d⁻¹). The TEs improved the anaerobic stability through an optimum alkalinity ratio (VFA/TIC < 0.3) and suppressed the accumulation of volatile fatty acids. Mono digestion of husks and pulp are prone to lack Mo, Zn, Ni and Fe in long-term anaerobic fermentation. Further studies on co-digestion of husk/pulp with animal manure and dry fermentation helps to efficiently use this biomass resource.

Keywords: Biogas / Coffee husk / Coffee pulp / Trace element / VFA/TIC

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1 Introduction

Coffee is grown in more than 70 countries worldwide, including 25 African countries. The global annual average coffee production during recent years (2013–2016) was about 9 million tons. Ethiopia is the largest coffee producer in Africa, and fifth in the world. The average green bean production from 2013 to 2016 was 395 000 tons per year [1].

Coffee cherries are picked from trees and processed to green beans following either the dry or the wet processing method [2]. For the dry method, the cherries are dried for 3 to 4 weeks after picking, before the outer fruit flesh is mechanically separated from the bean as dry husk, which is comprised of the pulp, mucilage and parchment [3]. For the wet method, the red cherries

are processed to separate the pulp as one fraction, the mucilage as the second fraction and the parchment as the final one [4]. The husk makes up for about half the weight of dry coffee cherries, while the pulp accounts for two-fifth of the fresh cherry [5, 6].

In traditional post-harvest approaches, by-products along the processing line are considered as waste and are dumped into the environment. In contrast, the biomass value web approach promotes the use of all parts of the crop in order to increase resource use efficiency [7]. However, until now, coffee by-products do not provide significant economic benefits for growers and hosting communities [8], and coffee processing facilities continue to dispose the residues into the environment instead of using them as a resource [9]. The wet processing method in particular generates organic pollutants rich in sugars and proteins [10, 11]. Thus, the by-products are causing severe environmental degradation, particularly in coffee growing and processing areas [12].

The methane yield of coffee husk and pulp in anaerobic digestion has been reported by several researchers [13–16], and the results indicate that these residues may have better performance compared to other agricultural residues [2, 6, 17, 18]. The

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Abbreviations: DOE, Days of experiment; OLR, Organic loading rate; TE, Trace element; TIC, Total inorganic carbon; VFA, Volatile fatty acid; VFA/TIC, Alkalinity ratio

authors determined the methane yield of husk and pulp at 159 and 245 L kg⁻¹ VS, respectively. However, to authors' knowledge, the biogas process has not yet been applied for coffee husk and pulp treatment on an industrial scale.

Volatile fatty acids (VFAs) are short-chain organic acids (C2–C6) produced by acidogenic bacteria and are intermediates formed during anaerobic fermentation of complex organic materials [19, 20]. The concentration of VFAs in a digester implies the health of the anaerobic process. The major components of the VFAs are acetic, propionic and butyric acids. Failure in the symbiotic interaction between the acidogenic and methanogenic microorganisms lead to a lower pH and an accumulation of VFAs. Besides other chemical and environmental factors, limited nutrient supply induces higher production of acetate and hydrogen, which leads to an accumulation of propionic and butyric acid due to extreme hydrogen partial pressure [19].

In addition to other parameters, such as OLR, alkalinity, concentration of toxic compounds, etc., the performance and stability of anaerobic digesters is highly affected by the availability of macro- and micronutrients. The macronutrients (N, P, K, Na, Ca, and Mg) are governing the biological processes of the digestion system, as well as the quality of the digestate as fertilizer. Micronutrients or trace elements (TE) (Fe, Zn, Mn, B, Co, Ni, Cu, Mo, Se, Al, W and V) are crucial for the growth and metabolism of anaerobic microorganisms, and a deficiency in TEs leads to a reduced methane production [21]. For example, biogas plants operated with maize silage as single substrate exhibited a consistent decline in biogas production and an increased process instability over time [22]. However, long-term biogas production from a single substrate (mono-digestion) can be stabilized by TE supplementation, thus avoiding acidification of fermenters and process failure [23, 24].

Mono-digestion of energy crops and agricultural residues can be prone to important TE deficits, leading to a reduced methane production. Digesters supplemented with TEs exhibited improved methane yield and system stability [22, 25–28]. Application of TEs suppresses the accumulation of volatile fatty acids (VFA), and significantly increases the methane content, allowing for a higher substrate feeding rate [23, 29]. Preißler et al. [30] indicated that the co-digestion of manure can reduce the TE deficiency and stabilize biogas production process from energy crops.

The organic loading rate (OLR) of the anaerobic reactor significantly influences the performance and stability of digesters, since a higher OLR can result in excessive accumulation of VFAs [31, 32]. A higher OLR favors bacterial activity in hydrolysis/acidogenesis, rather than in the methanogenesis phase, hence leading to an accumulation of VFAs [33]. Considering the reactor productivity, OLR of digesters is reached, when the methane yield per unit volume of digester is highest, while the anaerobic process is stable. The accurate determination of the degradation behavior of substrates requires continuous fermentation trials. Methane productivity increases linearly along with the increase in OLR until the optimum methane productivity is reached, beyond which the digester could be considered overloaded [34]. To the authors' knowledge, the effect of TE supplementation on anaerobic performance and stability have not yet been investigated on coffee husk and pulp operating in continuous assay with increasing organic loading rate.

The objective of this study was to investigate the effects of TE supplementation, comprising Fe, Ni, Zn, Co, Mn, Mo, Se, W and B, on the continuous mesophilic digestion of coffee husk and coffee pulp focusing on (i) stability and performance of the anaerobic digestion process and (ii) methane productivity and optimum OLR.

2 Materials and methods

2.1 Materials/substrates

The coffee husk and pulp were collected during the harvest season 2014/15 from Gomma-2 estate coffee farm (7°55'16.32"N, 36°37'06.62"E) about 400 km South-West of Addis Ababa, Ethiopia. Coffee pulp was collected right after pulping of the cherries on consecutive processing days in November 2014. The pulp then was spread for sun-drying on a plastic sheet for 3–4 days. The pulp was mixed from time to time to assure even drying. Coffee husk was collected from the same farm without a need of further drying. Both, the pulp and husk were ground in a mortar and milled with a grain mill and then passed through a 1 mm sieve. Afterwards the samples were packed in 2 kg volume polyethylene bags and shipped to the University of Hohenheim, Germany. The samples were stored in a cool and dry place until further use.

2.2 Anaerobic digestion trials

The experiments were performed in the biogas laboratory of the State Institute of Agricultural Engineering and Bioenergy, University of Hohenheim. Ten horizontal cylindrical digesters (diameter = 200 mm, length = 685 mm) made of stainless steel and with a volume of 20 L (working volume 16 L) each were operated as continuous stirred tank reactor (CSTR) according to VDI guideline 4630 [34] as illustrated in Fig. 1 by Haag et al. [35]. Substrate was fed into one side of the digester and the digestate was pushed out at the opposite side. The digester was stirred with an electric driven horizontal agitator for 80 s with a 180 s break. Digesters were heated to 40 ± 1°C with water circulating through a metal jacket. The biogas from each digester was continuously discharged through a gas outlet and collected in a gas bag after passing through a gas wash bottle. The amount of biogas and its composition in terms of CH₄ and CO₂, was measured daily at pre-set times at standard conditions (P = 101.325 kPa, T = 273.15 K). The internal temperature of the digester was measured with an automatic computer-interfaced system. Initially all digesters were filled with 16 L of inoculum obtained from a full-scale, maize and grass silages based biogas research plant at the University of Hohenheim [36] and run for two weeks without feeding substrate to degas and adapt microorganisms with the environment.

The experiment was conducted in five treatments with duplicates (Table 1). These were husk with and without TE supplement, pulp with and without TE supplement and control (pure inoculum). The control was used to correct gas production from the inoculum. Substrate feeding was started with an OLR of 2.5 kg VS m⁻³ d⁻¹ (HRT = 40 d) and was

Figure 1. Continuous stirred tank reactor (CSTR) adapted from Haag et al. (2015).

Table 1. Daily input materials (X) for different treatments of digesters

Treatment	Daily input materials			
	Water	Husk	Pulp	TE
Blank	X	–	–	–
Husk	X	X	–	–
Husk + TE	X	X	–	X
Pulp	X	–	X	–
Pulp + TE	X	–	X	X

Table 2. Organic loading rate (OLR) and hydraulic retention time (HRT) of digesters during corresponding days of experiment (DOE)

DOE (d)	OLR (kg VS m ⁻³ d ⁻¹)	HRT (d)
0–28	2.5	40.0
29–49	3.0	33.3
50–63	3.5	28.6
64–84	4.0	25.0
85–98	4.5	22.2
99–112	5.0	20.0
113–126	5.5	18.2
127–140	6.0	16.7

increased stepwise by 0.5 kg VS m⁻³ d⁻¹ up to 6 kg VS m⁻³ d⁻¹ (HRT = 16.7 d). Digesters were loaded with an OLR of 2.5 kg VS m⁻³ d⁻¹ for 28 days of experiment (DOE), and an OLR of 3 and 4 kg VS m⁻³ d⁻¹ for 21 DOE each. Other OLRs (3.5, 4.5, 5.0, 5.5 and 6.0 kg VS m⁻³ d⁻¹) lasted for 14 DOE each (Table 2). Substrates were mixed with tap water prior to feeding to adjust 10% VS content on fresh weight basis.

The degree of degradation (η_{VS}) was predicted according to VDI [34] by the mass ratio of the produced biogas (m_{biogas}) to the mass of VS fed (m_{VS}) to the digester:

$$\eta_{VS} = \frac{m_{biogas}}{m_{VS}} \cdot 100\% \quad (1)$$

$$m_{biogas} = V_{biogas} \times \left(\frac{M_{CO_2} \cdot c_{CO_2} + M_{CH_4} \cdot c_{CH_4}}{22.4 \cdot 100} \right) \quad (2)$$

Where V_{biogas} is the volume of biogas produced on dry gas basis, M_{CO_2} and M_{CH_4} is the molar mass of CO₂ and CH₄, respectively and c_{CO_2} , c_{CH_4} are CO₂ and CH₄ concentrations in the biogas, respectively.

2.3 Trace element

Trace element (TE) solution was composed of Cu (4050 mg kg⁻¹), Ni (5000 mg kg⁻¹), Zn (9000 mg kg⁻¹), Fe (27156 mg kg⁻¹), Co (2500 mg kg⁻¹), Mn (4800 mg kg⁻¹), Mo (2800 mg kg⁻¹), Se (720 mg kg⁻¹) and B (6600 mg kg⁻¹). (Schaumann Bioenergy GmbH, Wahlstedt, Germany). For the first 112 days of the experiment (DOE 0–112, first phase of TE application) the daily TE dose was 0.24 g per kg DM added. Commencing from DOE 113 (DOE 113–140, second phase TE application), the TE dose was doubled to 0.48 g kg⁻¹ d⁻¹ and Fe was supplied separately. The Fe solution was prepared by dissolving Fe₂O₃·6H₂O in deionized water and was applied as a daily dose of 6.81 mg Fe per L for husk substrate and 2.06 mg Fe per L for pulp substrate.

2.4 Analytical methods

The determination of ash and dry matter (DM) in % of fresh matter (FM) and volatile solids (VS) content in % of DM was carried out in accordance to DIN EN 14775, DIN EN 12880 and DIN EN 12879, respectively. The crude fiber (XF), acid detergent lignin (ADL), acid detergent fiber (ADF), and neutral detergent fiber (NDF) content was determined following Gerhardt fiber bag system methods. The nitrogen free extracts (NfE) were calculated by difference on dry mass basis.

The volatile fatty acids (VFAs) were determined by gas chromatography (GC). Thus 1 g of liquid sample material was weighed on an analytical balance in a 10 mL Erlenmeyer flask.

In order to stop further bacterial degradation of the fatty acids, the samples were acidified with 1 mL of orthophosphoric acid (17%) so that the pH-value fell into a strong acidic range of about pH 2.0. In addition, 1 mL of n-methyl-valeric acid was pipetted to each sample. The addition of this fatty acid, which does not occur naturally and is also referred to as the internal standard, serves to avoid possible injection errors in the subsequent GC analysis and to quantify the fatty acids contained in the samples. The samples were then filled with distilled water up to 10 mL and were shaken well (dilution 1:10). Approximately 2 mL of the solution were added to an Eppendorf tube and centrifuged with an ultra-centrifuge (Eppendorf Centrifuge 5415 D, Eppendorf, Hamburg, Germany) at about 13,200 RPM for 15 minutes. The supernatant of the samples was pipetted and transferred to a GC (Shimadzu, GC 2010plus with AOC-20i auto sampler with FID detector, Shimadzu, Munich, Germany). Individual volatile fatty acid contents (acetic acid, propionic acid, n- and iso-valeric acid, n- and iso-butyric acid and caproic acid) were determined after calibration with standard solutions.

The TE content of the sample was analyzed at Schumann Bioenergy (GmbH) laboratory, using inductively coupled plasma optical emission spectroscopy (ICP-OES). The sample was prepared by adding 10 mL of nitric acid and 2 mL of hydrogen peroxide and was dried at 103°C. Subsequently it was heated for 20 min and boiled for another 20 min. After cooling down to room temperature the sample was transferred to a 100 mL volumetric flask and distilled water was added to a level and then tested in a spectrometer. The concentration of the elements in the sample was determined from the wavelength of the optical emission.

2.5 The alkalinity ratio (VFA/TIC)

The ratio of the volatile fatty acids to total inorganic carbon (VFA/TIC), which is equivalent to FOS/TAC in German, is an alkalinity ratio at two-point titration measurement. About 50 g of the sample material was weighed in centrifuge bottles on an analytical balance and centrifuged at 5000 RPM (Z 323 K centrifuge, Hermle, Wehingen, Germany). Then 10 mL of the supernatant was mixed with 30 mL of distilled water in 100 mL glass beaker and placed in the auto-sampler (814 sample processor – Metrohm, Filderstadt, Germany) of a titrator (785 DMP Titrino, Metrohm, Filderstadt, Germany). After calibrating the pH electrode with pH 4.0 and 7.0 buffers, the samples were first titrated with 0.1 N sulfuric acid to pH 5.0 and then further to pH 4.4. The volume of sulfuric acid consumed to reach the respective pH values is equivalent to the amount of volatile fatty acids (VFA) and the amount of total inorganic carbon (TIC). To predict the TIC, the pH is titrated from an initial sample pH to 5.0, and the titration further continued from 5.0 to 4.4 for the VFA content. VFA and TIC contents were calculated as:

$$TIC = \frac{\text{Total volume (ml)}}{\text{Sample volume (ml)}} \cdot V_{TIC} \cdot 250/2 \quad (3)$$

$$VFA = \left(\frac{\text{Total volume (ml)}}{\text{Sample volume (ml)}} \cdot V_{VFA} \cdot 1.66 - 0.15 \right) \cdot 500/2 \quad (4)$$

Where, V_{TIC} is the volume of 0.1N H_2SO_4 consumed to titrate from an initial pH to a pH of 5.0 and V_{VFA} is the volume of 0.1N H_2SO_4 consumed to titrate from pH 5.0 to pH 4.4. The sample volume is the volume of supernatant (i.e. 10 mL), and the total volume is the volume of water plus supernatant (i.e. 40 mL).

The ratio of VFA to TIC provides information on the stability of the fermentation process and a ratio of ≤ 0.3 is recommended for a stable anaerobic fermentation [23].

Determination of ammonia/ammonium nitrogen was performed by mass analysis. About 3 g of a sample were weighed directly into 250 mL Kjeldhal flasks. The sample glasses were inserted into a Vapodest device (Vapodest 50S, Gerhardt, Koenigswinter, Germany). The Vapodest performs distillation by applying 32% sodium hydroxide to convert the ammonium in the sample into gaseous form, which is then liquefied again by distillation and collected in 2% boric acid. The pH of the boric acid thus changed from 4.6 to 7.5–8.0 as measured with a pH electrode immersed in the solution. After completing the distillation, the boric acid solution was titrated back to its starting pH with 0.1 molar hydrochloric acid (HCl). The amount of HCl was recorded. The quantity of HCl used corresponds to the amount of ammonium contained in the sample. The evaluation is made by the software Vapodest Manager, (Vapodest 50S, Gerhardt, Koenigswinter, Germany). The same procedure and devices were used to measure the total nitrogen (Kjeldahl nitrogen) except that the procedure started with the digestion of the sample with 20 mL of concentrated sulfuric acid and two tablets of catalysts (Kjeltabs CX consisting of 5 g of K_2SO_4 and 0.5 g of $CuSO_4 \cdot 5H_2O$), at 400°C for about 90 min.

2.6 Statistical analysis

The statistical analyses were performed using the statistical software SPSS 23 and Origin Pro-2016 to determine significant differences in the mean results of trials and to produce graphs, respectively.

3 Results and discussion

3.1 Characteristics of coffee husk and pulp

The bio-chemical properties of the pulp and husk show certain differences (Table 3). The VS content of the husk was higher than that of the pulp. The amount of cell wall components (cellulose, hemicellulose and lignin) was also higher in the husk (64.0%) than in the pulp (55.6%). Crude fiber content (39.2%) in the husk was significantly higher compared to the pulp (24.8%). Because the husk includes the parchment, which has significantly more cell wall components (32.2%) than any other parts of coffee by-products. The cell wall contents (the cellulose and lignin) of both the husk and pulp were comparable to other common agricultural residues [37, 38]. The higher lignin and crude fiber content in the husk could adversely influence the methane yield. Substrates with higher contents of lignin and crude fiber often exhibit lower biogas production [39]. Methane is mainly produced from cellulose for a longer fermentation time

Table 3. Proximate and bio-chemical analysis of substrates; values in % DM unless stated (mean $\pm$ std, $n = 3$)

	Pulp	Husk
DM (%FM)	92.30 $\pm$ 0.05	93.50 $\pm$ 0.04
VS	88.30 $\pm$ 0.16	92.80 $\pm$ 0.05
Crude ash	11.69 $\pm$ 0.16	7.15 $\pm$ 0.05
Crude protein	14.06	11.06
Crude fat	0.99 $\pm$ 0.07	1.45 $\pm$ 0.03
N ^a	48.47	40.41
Crude fiber	24.78 $\pm$ 1.20	39.92 $\pm$ 0.05
Hemicellulose	8.50 $\pm$ 1.42	14.50 $\pm$ 1.04
Cellulose	31.60 $\pm$ 1.74	32.00 $\pm$ 1.52
Lignin	15.50 $\pm$ 1.64	17.50 $\pm$ 1.55

^a) Non fiber carbohydrate**Table 4.** Elemental analysis of husk and pulp and recommended range of trace elements (TE) for stable anaerobic fermentation; values on DM basis

Element	Elemental analysis (mg kg ⁻¹)		Recommended range of TE ^a (mg kg ⁻¹)	
	Pulp	Husk	min	max
Mn	159.0	87.9	100	1500
Zn	13.2	3.4	30	300
Co	0.7	0.5	0.4	5.0
Mo	0.16	0.07	1.0	6.0
Fe	1196	402	1500	3000
Ni	1.16	1.20	3.0	16
Se	<0.05	<0.05	0.2	2.0
W	<0.05	<0.05	0.1	30

^a) Oechsner et al. (2008)

(weeks) than the hemicellulose, which is easily degradable within a few days [40].

Table 4 shows the elemental analysis of the substrates for trace metals and shows that the pulp was particularly richer in Fe, Mn and Zn than the husk. The trace metal contents in husk and pulp were analyzed and compared with the optimal range for a stable anaerobic fermentation as suggested by Oechsner et al. [41]. The results clearly indicated that both, the husk and pulp had deficiencies in Mo, Zn, Fe, Ni, Se and W contents, but had fairly sufficient contents of Mn and Co. The amount of Mo detected in pulp and husk was only 16 and 7% respectively, of the minimum requirement for a stable anaerobic process. Both substrates were seriously deficient in Mo compared to other TEs detected in the substrates. The amount of Zn was 44 and 11% of the requirement in the pulp and husk substrates, respectively. Fe, the only trace metal required in a higher amount, was also detected in pulp and husk at a deficiency of 20 and 73%, respectively. The Ni, W and Se contents were 40, 50 and 46% of the minimum requirements respectively, both in husk and pulp equally.

3.2 Gas production and quality

Figure 2A shows the average daily SMY from pulp and husk with and without TE supplement during 140 DOE while

Figure 2. (A) Specific methane yield (SMY) and (B) methane productivity of digesters corresponding with an increasing organic loading rate (OLR) with and without trace element (TE) supplement versus day of experiment (DOE). The vertical broken lines indicate the change of TE dose (STP: 273.15 K, 101.33 kPa) (C) Specific methane yield (SMY) for each step of the organic loading rate (OLR); with and without trace element (TE) supplement (mean $\pm$ std, $n = 10$).

increasing the OLR from 2.5 kg m⁻³ d⁻¹ (HRT = 40 d) to 6.0 kg m⁻³ d⁻¹ (HRT = 16.7 d). An independent sample *t*-test was conducted to compare the daily SMY during the first 112 days (while TEs were 0.24 g kg⁻¹) from husk and pulp with and without TE supplement. There was no significant difference in the SMY of husk with TE (148 $\pm$ 24 L kg⁻¹) and without TE supplement (145 $\pm$ 23 L kg⁻¹). In the same period, there was also no significant difference in the SMY of pulp with TE (206 $\pm$ 40 L kg⁻¹) and without TE supplement (205 $\pm$ 40 L kg⁻¹). The results

indicated that the amount of TEs supplemented was not sufficient to achieve a significant difference on the SMY. This could be attributed to the higher TE content in the inoculum despite a decreasing effect due to the dilution through daily substrate feeding. However, during the second phase of the TE dose and Fe application, DOE 113–140, there was a significant ($p < 0.00001$, $\alpha = 0.05$) effect on the SMY of pulp and husk supplemented with TEs, compared to digesters not supplemented with TEs. The effect of TE supplements was more visible during the last 4 weeks of the trial.

The specific methane productivity, which is the average methane yield per unit of active working volume of digester and day, is shown in Fig. 2B. For the first 112 DOEs (with an OLR ranging from 2.5 to 4.5 kg VS $\text{m}^{-3} \text{d}^{-1}$) there was a slight difference in the methane productivity between digesters supplemented with TEs and without for both pulp and husk substrates. At an OLR of 5 kg VS $\text{m}^{-3} \text{d}^{-1}$, pulp without TEs exhibited a maximum methane productivity of 0.939 $\text{m}^3 \text{m}^{-3} \text{d}^{-1}$, while at the same OLR the TE supplemented pulp showed an increase in methane productivity of 14.6%. During the following DOEs, the methane productivity from pulp digesters declined steadily and eventually reached 0.434 $\text{m}^3 \text{m}^{-3} \text{d}^{-1}$ at the end of the trial, which is similar to the productivity at an OLR of 2.5 kg VS $\text{m}^{-3} \text{d}^{-1}$. However, the TE supplemented pulp digesters showed an increasing trend and reached 1.243 $\text{m}^3 \text{m}^{-3} \text{d}^{-1}$ at a maximum OLR of 6.0 kg $\text{m}^{-3} \text{d}^{-1}$. The highest methane productivity from husk with and without TE supplement was 0.890 $\text{m}^3 \text{m}^{-3} \text{d}^{-1}$ and 0.793 $\text{m}^3 \text{m}^{-3} \text{d}^{-1}$, respectively both at an OLR of 6.0 kg $\text{m}^{-3} \text{d}^{-1}$. Thus, the second phase of TE and Fe application showed an increase in methane productivity from 4.4% at DOE 112 up to 12.2% at DOE 140 compared to husk digesters without TEs. The methane content in the biogas coupled with an acceptable volatile fatty acid concentration and alkalinity ratio (VFA/TIC) implied that the digesters could even be loaded above an OLR of 6 kg $\text{m}^{-3} \text{d}^{-1}$ with husk and pulp supplemented with TEs.

Figure 2C shows the mean SMY of digesters when increasing the OLR of substrates from 2.5 to 6.0 kg VS $\text{m}^{-3} \text{d}^{-1}$. The mean SMY of digesters for each OLR was calculated from the SMY produced within the last 10 days of each OLR. The mean SMY of husk ranges from 132.5 $\pm$ 4.9 to 174.1 $\pm$ 7.9 L kg^{-1} VS at an OLR of 6 and 3.0 kg $\text{m}^{-3} \text{d}^{-1}$, respectively and from 140.6 $\pm$ 16.7 to 177.3 $\pm$ 6.4 L kg^{-1} VS at an OLR of 2.5 and 3 kg $\text{m}^{-3} \text{d}^{-1}$, respectively for husk with TEs. Similarly, the mean SMY of pulp with TE ranges from 206.2 $\pm$ 9.1 to 224.8 $\pm$ 7.3 L kg^{-1} VS at an OLR of 4.5 and 3.0 kg $\text{m}^{-3} \text{d}^{-1}$, respectively. Pulp (without TEs) showed the highest SMY 227.7 $\pm$ 6.2 L kg^{-1} VS at an OLR of 3.0 kg $\text{m}^{-3} \text{d}^{-1}$. There was no statistically significant ($p = 0.104$) difference in the mean SMY from pulp supplemented with TEs while increasing the OLR from 2.5 to 6.0 kg VS $\text{m}^{-3} \text{d}^{-1}$. However, husk digesters supplemented with and without TEs had a significantly higher SMY of 177.3 and 174.4 L kg^{-1} VS, respectively; at an OLR of 3 kg VS $\text{m}^{-3} \text{d}^{-1}$. No significant difference in SMY of pulp digesters was observed for an OLR of 2.5–5.0 kg VS $\text{m}^{-3} \text{d}^{-1}$. The effect of TE application on the mean SMY of same substrate digesters, with and without TEs, was compared based on the OLR steps. Thus, for OLRs of 2.5–4.5 kg VS $\text{m}^{-3} \text{d}^{-1}$ there was no statistically significant difference in the mean SMY from both husk and pulp digesters with and

without TEs. However the effect of TE application was significant ($p < 0.01$) for both pulp and husk digesters at OLRs of 5.0–6.0 kg VS $\text{m}^{-3} \text{d}^{-1}$.

3.3 N_{tot} and $\text{NH}_4\text{-N}$

Basically, the $\text{NH}_4\text{-N}$ represents ammonium ions (NH_4^+) and as free ammonia (NH_3), the latter being responsible for process inhibition above certain limits depending on the prevailing temperature and pH of digesters. Methanogens are least tolerant for ammonia inhibition among anaerobic microorganisms [42]. Both, husk and pulp showed a continuous decline from 6170 mg kg^{-1} FM in N_{tot} and 3195 mg kg^{-1} FM in $\text{NH}_4\text{-N}$ throughout the trial period, regardless of the increasing OLR. The elevated contents of the N_{tot} and $\text{NH}_4\text{-N}$ during the first few DOEs could be associated with the start-up inoculum. At the end of the trial, N_{tot} and $\text{NH}_4\text{-N}$ contents of the husk with and without TEs were 2220 mg kg^{-1} FM and 320 mg kg^{-1} FM respectively; and for pulp with and without TEs it was 2775–2860 mg kg^{-1} FM and 465 mg kg^{-1} FM, respectively. The $\text{NH}_4\text{-N}$ content of digesters, which is an indicator for process stability, was well under the recommended inhibition threshold limits of 5000 mg kg^{-1} FM [43]. Ammonia inhibition was not a threat in the anaerobic fermentation of husk and pulp at mesophilic CSTR.

3.4 DM-VS degradability

The DM content of digestates from pulp with and without TEs was in a range of 5–6.7% while from husk it was 5.2–9.4%, throughout the trial period. The VS content in the husk and pulp with and without TE digestates was nearly 90 and 80%, respectively. Both the DM and VS content from pulp was essentially lower than that of the husk for the same substrates, irrespective of the TE supplement (Fig. 3A and B).

The average VS degradation efficiency (η_{VS}) in pulp was similar for an OLR of 2.5–4.5 kg $\text{m}^{-3} \text{d}^{-1}$ with and without TEs (Fig. 3C). However, the η_{VS} of pulp without TEs declined abruptly at an OLR of 5.0–6 kg $\text{m}^{-3} \text{d}^{-1}$, while pulp with TEs remained steady. In the same OLR range, TE supplemented husk had a moderately higher (4.1–7.2%) η_{VS} compared to the husk without TEs. The η_{VS} in husk with and without TEs was more stable than in pulp. Generally, the η_{VS} of pulp was higher than that of husk, which could be attributed to the lower lignin and crude fiber content.

3.5 pH value

In the course of the trial, the pH of digesters gradually decreased from the initial value of 7.90 while increasing the OLR (Fig. 3D). The difference in pH between husk with and without TE supplement was very slight. However, pulp, particularly starting from an OLR of 5.5 kg $\text{m}^{-3} \text{d}^{-1}$ onward, showed a significant decline and the average pH at the highest OLR of 6 kg $\text{m}^{-3} \text{d}^{-1}$ was 6.42 $\pm$ 0.52, which is below the threshold limit for methane forming bacteria. At the highest OLR of 6 kg $\text{m}^{-3} \text{d}^{-1}$, pulp with TEs and husk with and without TEs exhibited an average pH

Figure 3. (A) Dry matter content (DM) and (B) volatile solids (VS) versus day of experiment (DOE); (C) volatile solids degradation efficiency and (D) pH value versus organic loading rate (OLR). The vertical broken line shows the change of TE dose.

of 7.42 ± 0.08 , 7.25 ± 0.02 and 7.19 ± 0.07 , respectively. The optimum pH interval for methane formation is 7.0–8.0 [23].

3.6 Volatile fatty acids (VFA)

Figure 4 shows the total volatile fatty acid (VFA_T) contents (acetic, propionic, n-butyric, iso-butyric, n-valeric, iso-valeric and capronic acids) as well as the individual acetic and propionic acid contents. In the first 112 DOE, while OLR was at 2.5 to 5 kg m⁻³ d⁻¹, there was no significant difference in the acetic and propionic acid contents between husk digesters with and without TEs. However, in the subsequent DOEs (OLR 5.0 to 6.0 kg m⁻³ d⁻¹) there was a clear difference between the variants with and without TEs, while the variants with TEs stayed constant. The variants without TEs showed an exponential increase in the acetic and propionic acid contents and exhibited 367 and 897 mg kg⁻¹, respectively by the end of the trial. Even butyric acid and valeric acid and their branched isomers were detected within the last 2 weeks but were not above the optimum value recommended for stable fermentation. Interestingly these higher VFA were not detected in TE supplemented husk at all.

The acetic and propionic acid content of pulp remained steady within the first 84 DOEs (OLR 2.5 to 4 kg m⁻³ d⁻¹). In the period 85 to 112 DOE, the acetic acid increased from 81 to 137 mg kg⁻¹ and from 185 to 1874 mg kg⁻¹ in pulp with and without TE supplement, respectively. In the same period, the propionic acid content was recorded at 266 mg kg⁻¹, but was not detected in TE supplemented pulp. During DOEs 113–140, the acetic and propionic acid contents were increasing significantly as well and

reached values as high as 7958 and 3010 mg kg⁻¹, respectively. For the same period, butyric and valeric acid and their branched isomers were detected in pulp, with values far above the recommended maximum threshold values of 50 mg kg⁻¹; but were hardly detected in TE supplemented pulp digesters [44]. On the last DOE, the VFA_T and acetic acid contents in pulp without TEs were higher than in pulp with TEs by a factor of 75 and 56 respectively; while in husk without TEs it was higher than in husk with TEs by a factor of 17 and 12, respectively. The TE application, particularly during DOEs 113–140, effectively suppressed the accumulation of VFA, both in pulp and husk. For a stable anaerobic digestion, maximum concentrations of VFA is around 4.000 mg L⁻¹, where the individual maxima of acetic acid and propionic acid are 3.000 and 1.000 mg L⁻¹, respectively. The butyric acid and valeric acid concentration is recommended to be less than 50 mg L⁻¹ [43].

3.7 Alkalinity ratio (VFA/TIC)

The intermediate alkalinity (VFA) and partial alkalinity (TIC) of digesters with and without TE supplement is shown in Fig. 5A. The acid buffering capacities (TIC) of all digesters were continuously declining corresponding to an increased OLR, and are thus more susceptible to pH changes while accumulating volatile fatty acids. The intermediate alkalinity (VFA), was fairly the same in TE supplemented pulp and husk (with and without TE) digesters. However, the VFA in pulp (without TE) starts increasing at an OLR of 5.0 kg m⁻³ d⁻¹. Both husk and pulp digesters supplemented with TEs exhibited a VFA/TIC ratio well

Figure 4. Total volatile fatty acid (VFA_T), acetic acid and propionic acid content from (A) pulp and (B) husk digesters with and without TE supplement versus day of experiment (DOE).

Figure 5. Courses of (A) Intermediate alkalinity (VFA) and partial alkalinity (TIC) and (B) alkalinity ratio (VFA/TIC) of samples from pulp and husk digesters versus day of experiment (DOE). The horizontal broken line in (B) shows the recommended VFA/TIC ratio (=0.3) for a stable anaerobic fermentation.

below the recommended value of 0.3 throughout the trial period. Pulp containing digesters without TEs showed a VFA/TIC ratio above 0.3 from DOE 105 on (while the OLR was 5 kg m⁻³ d⁻¹). On the other hand, husk digesters (without TEs) had a VFA/TIC value just under the threshold limits (Fig. 5B). The application of TE suppressed accumulation of VFA in the pulp digesters, as observed during the last weeks of fermentation, led to stable VFA/TIC values. Besides this, the VFA/TIC values from husk digesters indicate the possibilities for higher OLRs without significant system disruption and gas production.

3.8 Trace element analysis

Figure 6 shows the TE concentrations (Fe, Ni, Zn, Co, Mn, Mo, Se and W) of effluents measured for each step of the OLR. The concentration of trace metals in the digester exhibited a declining trend, which suggests dilution of the inoculum. Elemental analysis of pulp and husk substrates indicated a lack of trace metals (Mo, Zn, Fe, Ni, Se and W) compared to the recommendation by Oechsner et al. [41]. The Fe content of pulp with and without TEs decreased gradually but was not below the recommended threshold of 1500 mg kg⁻¹ (Fig. 6A). The Fe content in the fresh pulp was only about 80% of the minimum requirement. In contrast, husk digesters showed a distinct decline from DOE

28 on and continued below the minimum Fe threshold. The second phase of TE application (DOE 113–140) increased the Fe contents for TE supplemented husk significantly. Husk showed serious deficiencies in Fe compared to pulp. This indicates that mono-digestion of husk might pose a severe deficiency in Fe contents and thus consequently impair the bio-methane yield. The Ni and Mo contents (Fig. 6B and F) were above the minimum threshold limits of 3 and 1 mg kg⁻¹ respectively in TE supplemented pulp and husk, while substrates (without TE) remained below the threshold. The second phase of TE supplementation increased the Ni and Mo contents. The fresh pulp and husk had only 16 and 7% of the minimum threshold for Mo respectively, and about 40% of Ni. Pobeheim et al. [22] demonstrated a 30 and 15% higher methane yield when applying Ni and Co on maize silage, respectively.

During the last days of the trial, the Zn content reached the minimum threshold of 30 mg kg⁻¹ DM. The TE supplement was not significant in improving the Zn contents in the digester. Nevertheless, the inoculum was rather rich in Zn, as evident during the first days of the trial. (Fig. 6C). The Se content in TE supplemented digesters was higher than for those without TEs. However, digesters without TEs were not below the minimum threshold at the end of the trial (Fig. 6G). Despite a declining trend in W content (Fig. 6H), pulp still had a higher W content than husk. Both, husk and pulp had no deficiency in Co and Mn

Figure 6. Courses of individual trace element (TE) concentrations (DM basis) versus day of experiment (DOE). Horizontal broken lines indicate the minimum TE requirements for a stable biogas fermentation, whereas the vertical broken line shows the boundary between the first and second phase of TE application, values indicate mean of duplicates.

(Fig. 6D) and (Fig. 6E), since they are sufficiently available in the fresh substrate. Unlike other trace metals, the TEs increased the concentration of Co in digesters.

In general, digesters without TE lack Fe, Ni, Zn and Mo particularly during the last days of the trial. In contrast, Co, Mn, Se and W were measured above the minimum thresholds. Thus none of these metals could be limiting factors for the anaerobic processes. The second phase of TE application (DOE 113–140), had a positive effect on the biogas performance and stability of

digesters as evidenced by suppressed VFA and alkalinity ratios, despite an increased OLR.

4 Concluding remarks

In this study, the anaerobic performance of coffee pulp and husk was investigated, while increasing the organic loading rate with and without trace element (TE) supplements. Coffee husk and

pulp have a promising biogas yield potential and supplementation with TEs has an important effect on the stability of the fermentation process, proven by a suppressed content of short chain volatile fatty acids. Moreover, the TEs allow a higher organic loading rate (OLR) and hence, a higher biogas production rate could be achieved. Mono-digestion of husk and pulp may also be an option but with a lower OLR. We recommend future research on husk and pulp to (i) investigate the anaerobic performance at OLRs above $6 \text{ kg VS m}^{-3} \text{ d}^{-1}$ (ii) optimize co-digestion with animal manure to meet the TE requirements and (iii) examine dry fermentation.

Practical application

Mono digestion of agricultural residues is prone to anaerobic instability and lower performance due to the lack of important trace elements in the substrate. Coffee husk and pulp are the major by-products generated from primary coffee processing. The by-products have virtually no economic use, but are creating environmental problems. In this study, the anaerobic performance of husk and pulp is demonstrated in continuous stirred tank reactors at a high organic loading rate and supplementation with trace elements. The specific methane yield of husk and pulp in accordance to the increasing organic loading rate and trace element addition is presented. The ultimate stable organic loading rate of husk and pulp with and without trace element supplementation is indicated. Based on these results, coffee processing facilities can utilize their by-products through anaerobic fermentation for productive use.

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