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SPENT COFFEE GROUNDS FROM COFFEE VENDING MACHINES AS FEEDSTOCK FOR BIOGAS PRODUCTION

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Abstract

Large amounts of spent coffee grounds (SCG) are currently available all over the world due to the enormous increase in coffee consumption. This increase has in turn to be related to the even greater diffusion of coffee vending machines. The aim of this study was to evaluate the biomethanation potential (BMP) of SCG alone or in co-digestion with pig slurry (PS). Pig slurry was chosen because it is frequently utilized as feedstock for biogas production from agricultural waste. The raw material was obtained from the SCG-collecting tank of a commercial coffee vending machine. Compared treatments were: SCG, PS and SCG+PS. Depending on the treatment, each reactor (100 mL) contained: 1 g (2%) SCG volatile solids (VS) and 50 mL of hydration medium (in SCG) or PS (in SCG+PS); 50 mL of PS (in PS). A lab-prepared inoculum (10% v/v) was added to each reactor. Biogas production at 35 °C and composition were monitored until exhaustion of the anaerobic digestion (AD). The BMP of SCG was 290 mL CH₄ g⁻¹ VS, a value comparable to that of other substrates currently used as ingredients in anaerobic digestion. Using PS instead of hydration medium increased the CH₄ production per reactor. We conclude that SCG are a suitable feedstock for biogas production. Our in-batch results suggest also the potential for increasing biogas yields from pig slurry using spent coffee grounds as co-substrates, in continuous systems.

Key words: biomethanation potential, co-digestion, methane, pig slurry, spent coffee grounds

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1. Introduction

The coffee water extract is a beverage widely spread all over the world. The world coffee beans production in the 2017/18 biennium is estimated to be around 9.5 million tons (USDA, 2017). The European Union imports nearly 40% of the whole world coffee production, and this quantity is practically constant since 2012 (USDA, 2017). It is therefore not surprising that 17 of the 20 countries in the world with the highest per capita consumption of coffee are European (ICO, 2017). As the EU imports exclusively coffee beans (USDA, 2017), their roasting is carried out in the EU territory. Two kinds of waste are produced in the coffee roasting process: the residues of the roasting process and the solid residues from the

extraction process, the so-called “spent coffee grounds” (SCG). These residues can derive either from brewed or from espresso coffee preparation (in a 50 to 50 proportion; Cruz et al., 2012b). Regardless of the extraction procedure, the mean per capita consumption in the EU is 7 kg. With reference to the consumption data, Europe can be divided into 3 macro areas: the Mediterranean-Balkan area with an average consumption per capita of 5.4 kg, Central Europe (6.1 kg per capita, on average) and Northern Europe (9.4 kg per capita, on average). When taking into account the population of the various States, it can be concluded that SCG are widely available throughout Europe.

Currently, most of the SCG are disposed of in landfills, despite their high organic load (Franca and

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Oliveira, 2009). Spent coffee grounds are considered eco-toxic (Ciesielczuk et al., 2017) due to their high content in caffeine, tannins and polyphenols (Buerge et al., 2003; Mussatto et al., 2011). In recent years, several alternative uses of SCG have been evaluated, for example as ruminant feeds (Givens and Barber, 1986), or as ingredients of bakery goods in human nutrition (Martinez-Saez et al., 2017). Their use as crop fertilizers or soil amendments has also been suggested (Campos-Vega et al., 2015; Cruz et al., 2012a). Other authors have emphasized the interest of SCG as a source of bioactive molecules. According to Machado et al. (2012) SCG can be used as a feedstock for fungal strains capable to extract polyphenolic compounds (*Penicillium purpurogenum*, *Neurospora crassa* and *Mucor*). Other authors (Acevedo et al., 2013; Cruz et al., 2012b; Scully et al., 2016) report that remarkable amounts of chlorogenic acids and caffeine can be obtained from SCG, which are particularly requested by the food and pharmaceutical industry.

Spent coffee grounds can also be exploited for energy production. In fact, they can be directly burned (Silva et al., 1998), or pyrolysed (Li et al., 2014; Luz et al., 2018a), or they can be used as raw material for production of liquid fuels, such as bioethanol (Kwon et al., 2013; Mussatto et al., 2012) and biodiesel (Calixto et al., 2011; Kondamudi et al., 2008; Oliveira et al. 2008; Somnuk et al., 2017). However, these types of exploitation are involved in particulate emissions into the atmosphere (Kim and Choi, 2010).

Anaerobic digestion (AD) can represent an interesting alternative to direct combustion of SCGs or to their transformation into liquid biofuels. In the past, several authors suggested the possibility of obtaining biogas from SCG both in mesophilic (Lane, 1983) and in thermophilic (Kida and Sonoda, 1992) conditions, but the system, in both cases, did not have adequate long-term stability. More recently, Luz et al. (2017) obtained interesting results by feeding an anaerobic reactor with the liquid fraction deriving from the spent coffee filtration. To overcome the problem of digestion stability, Luz et al. (2018b) also proposed the hydrothermal carbonization of SGC as a pretreatment before anaerobic digestion. This pretreatment allowed a remarkable increase in CH₄ production. However, a longer lag phase duration was observed in the pre-treated samples. Moreover, the pre-treatment application requires an additional energy input, which can make the process economically unsustainable. Vitez et al. (2016) carried out mesophilic AD using SCG from a coffee shop as feedstock, without detecting any problems of process stability. The same authors however conclude that, although the biogas production by SCG is a viable solution, the limiting factor to its application is the lack of a system of collection and transport suitable for this kind of waste.

In recent years, in the EU and especially in Italy there has been an increase in the spreading of coffee vending machines. Currently there are in the EU 2.2 million of coffee vending machines (EVA, 2017),

installed mainly in public areas such as airports, railway stations, hospitals, universities, etc. The maintenance (supply and discharge of the SCG) is entrusted to the installer companies; this means that SCGs are collected and transferred to a single centre that belongs to a specific geographical area. It follows that, in the face of a widespread diffusion of coffee vending machines, there is a concentration of SCG in a few areas, and this can represent a solution to the problem of the collection and transport of SCG.

The aim of this study was to evaluate the biomethanation potential (BMP) of SCG collected from a commercial coffee vending machine, with particular attention to its possible use as co-ingredient in the AD of pig slurry, a feedstock frequently utilized in biogas production from agricultural waste.

2. Materials and methods

2.1. Feedstock

Spent coffee grounds were collected from the coffee vending machine at our Research unit. The operation of the machine is completely automatic: the roasted coffee beans are freshly ground at the time of delivery of the coffee to the customer, and the SCG are automatically transferred to a plastic bag placed in the lower part of the machine. A maintenance worker periodically picks up the full bag and takes it to the vending machine-producing company. The samples of SCG were collected on one of these occasions, and frozen until use. They were used as they are, without any pre-treatment or sterilization.

Fresh pig slurry was collected from the first collection tank of our experimental farm in S. Cesario sul Panaro (MO), after mechanical homogenisation, and it was used immediately after collection. Selected analytical characteristics of these materials are reported in Table 1.

2.2. Experimental design

Compared treatments were: SCG, PS and SCG+PS, with 3 replications, for a total of 9 reactors. Anaerobic digestion was carried out in batch, in mesophilic conditions (35 °C). Biogas volume and composition were determined for each reactor during the incubation period (3 months).

2.3. Inoculum preparation

The inoculum was prepared according to Vasmara et al. (2015), using pig slurry as raw material. The liquid fraction of pig manure after solid separation was used for this purpose. It was withdrawn from the main farm storage tank, at two-thirds depth. Pig slurry was mixed with hydration medium (sterilized phosphate buffered basal medium without energy sources, HM) in a 1:1 volume ratio, in modified atmosphere (N₂-CO₂, 80:20). This mixture was left to incubate at 35 °C, in strictly anaerobic conditions (degassing phase)

Table 1. Selected composition characteristics of the materials used in the experiment.

<i>Parameter</i>	<i>Unit</i>	<i>Pig slurry</i>	<i>Spent coffee grounds</i>
Total solids (TS)	%	1.42	47.35
Volatile solids	% FW	1.04	46.46
pH		7.2	6
Kjeldahl N	% FW	0.093	0.147
Total P	% FW	0.029	0.067
Organic carbon	% TS	39.48	50.61
Neutral Detergent Fibre (NDF)	g kg ⁻¹ TS	300	647
Acid Detergent Fibre (ADF)	g kg ⁻¹ TS	175	362
Acid Detergent Lignin (ADL)	g kg ⁻¹ TS	82	168
Crude Fat	% TS	6.00	9.08
Total polyphenols (Folin-Ciocalteu method)	% TS	0.134	0.200
Caffeine	mg kg ⁻¹ TS	n.d.	30.3

FW= fresh weight; n.d. = not determined

The inoculum was considered as ready for use when gas production had stopped, indicating the complete exhaustion of endogenous energy sources. At the end of the degassing phase it was centrifuged, and the pellet was resuspended in HM (inoculum to HM ratio: 10:1), in anaerobic conditions. A fixed volume of this suspension (10% of the liquid phase in the reactor, v/v) was used to inoculate each digestion reactor. As the inoculum did not produce CH₄ anymore when ready for use, it gave no contribution to the final amount of cumulated CH₄. The inoculum VS content was 5.72% FW.

2.4. Anaerobic digestion

Anaerobic digestion was carried out in 100-mL reactors (118.5 mL effective volume) according to Owen et al. (1979). The headspace of the reactors was gassed with 100% N₂ throughout the preparation steps before inoculation. Reactors were plugged with butyl rubber stoppers and aluminum seals and they were incubated at 35 °C for 91 days. During the incubation period they were randomly distributed on the incubator shelves. Biogas was collected by means of 100 mL glass syringes as described in Vasmara and Marchetti (2016). The incubation period was completed when there was no more biogas production in any of the reactors. Three reactors containing only inoculum and HM were also included as blanks. No methane production was detected in the blank reactors.

In this experiment, 3 digestion times were assumed as representative of the CH₄ production curve: the start of the linear phase of CH₄ production (4 d after inoculum); the time when all the reactors were in the linear phase of CH₄ production (14 d after the start of the incubation); and the time when all the reactors had joined the stationary phase, at the end of the incubation period (91 days). As the lag phase was practically lacking, the amount of CH₄ produced 4 and 14 d after the start of the incubation can be considered as an estimate of the rate of CH₄ production, whereas the cumulated amount of CH₄ at the end of the digestion period is identified as the maximum CH₄ production (Mmax). The BMP value is given by the

ratio of Mmax to the VS content of the substrate. Blanks did not produce any CH₄, therefore no CH₄ subtraction to the CH₄ production of the samples was needed.

2.4.1. Monodigestion conditions

Anaerobic digestion was carried out using SCG as substrate. The reaction mixture included 1 g of SCG VS in 50 mL HM. The initial pH of the mixture was adjusted to 7. pH adjustment at the desired level was made in each reactor before inoculation, with NaOH 32%, using a syringe equipped with a sterile filter (pore size 0.2 µm). Since in each reactor 1 g of SCG VS had been added, on the basis of the VS inoculum content the inoculum to substrate VS ratio in monodigestion was 0.29.

2.4.2. Co-digestion conditions

In co-digestion with pig slurry (SCG+PS), 1 g SCG VS was added in each reactor to 50 mL of non-sterilized pig slurry (0.5 g VS), for a total of 1.5 g VS per reactor. Since in each reactor 1.5 g of SCG VS had been added, on the basis of the VS inoculum content the inoculum to substrate VS ratio in co-digestion was 0.19.

Three reactors with 50 mL PS alone (0.5 g VS) were included as controls. The initial mean pH value was on average equal to 7. Since in each reactor only 0.5 g of PS VS had been added, on the basis of the VS inoculum content the inoculum to substrate VS ratio in the control reactors was 0.57.

Due to the solid matter contribution, the final volume of the SCG treatment, in monodigestion or in co-digestion with PS, was 53 mL, corresponding to a 6% increase of the overall feedstock suspension volume (feedstock + hydration medium).

2.5. Analytical methods

Methane concentration in the biogas was determined by means of a MicroGC Agilent 3000 gas-chromatograph, equipped with 2 columns: Molsieve and Plot U; detector: TCD. Carrier gas: argon. Methane volume was expressed in standard conditions

of temperature and pressure (STP; 273 °K and 760 mm Hg).

The total solid (TS), volatile solid (VS), organic C, Kjeldahl N, and total P contents and pH were determined in SCG and PS according to APHA (1992). Total solids and ashes were determined gravimetrically, after thermal treatment respectively in an oven at 105 °C at constant weight, and in a muffle furnace at 550 °C for 10 hrs. The VS content was calculated as the difference between TS and ashes. Organic C was determined with the dichromate reflux method. Total N was determined with the Kjeldahl apparatus. Total P was determined on ashes by colorimetry with ammonium molybdate, after solubilisation by means of HCl 1 N. pH was measured potentiometrically (dry matter: water ratio, 1:10; 2-h stirring and sedimentation). The fat content was determined gravimetrically, after extraction of 1 g TS by means of a Soxhlet extractor. Fiber fractions (neutral detergent fiber, NDF; acid detergent fiber, ADF; and acid detergent lignin, ADL), were determined on SCG and PS according to Van Soest et al. (1991). Caffeine was determined by spectrophotometry, at 271 nm (Salihović et al., 2014), using caffeine from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany) for the preparation of the standard solutions (0, 2, 4, 6, 8, 10 mg caffeine L⁻¹ distilled water). Caffeine was extracted from the SCG samples according to the method of Cruz et al. (2012b) with some modifications. Five hundred milligrams of SCG were suspended in 50 mL of distilled water and left to boil for 10 min in continuous stirring. The suspension was then centrifuged at 4000 rpm for 10 min. The supernatant was kept aside and the pellet suspended again in 50 mL distilled water, left to boil for 10 min in continuous stirring, and centrifuged. This procedure was repeated two more times. Eventually, the supernatants were filtered and combined into a single 200-mL volumetric flask. After cooling, the volume was adjusted at 200 mL. The samples were appropriately diluted before spectrophotometric analysis. Total polyphenols were determined colorimetrically, using the Folin-Ciocalteu reagent (Singleton and Rossi, 1965) and expressed as percent gallic acid per g of dry matter.

2.6. Statistical analyses

The analysis of variance was carried out using the SAS package procedures. The PROC MIXED procedure (Littell et al., 1996) was used to test the significance of the treatment effects on BMP and on the CH₄ volumes produced 4, 14 and 91 days (Mmax) after the start of the incubation. Multiple comparisons of the means were carried out using the SAS LSMEANS statement. Factor effects were considered significant at $P < 0.05$. The Tukey Honestly Significant Difference (HSD) at $P = 0.05$ was used to compare the treatment mean values.

3. Results and discussion

The CH₄ concentration in biogas produced from SCG during the whole incubation period (Fig. 1) was very similar, on average, to the CH₄ concentration obtained from PS ($41 \pm 1.9\%$ vs $44 \pm 1.5\%$, $n = 14$). The lowest value of CH₄ concentration for SCG was 18%, measured 4 days after the start of incubation, and the highest CH₄ concentration was 58%, measured at the 14th day of incubation. These values are in agreement with those reported by other authors (Luz et al., 2017; Vitez et al., 2016).

Even though the adopted inoculum to substrate ratio was relatively low when compared to those usually reported in the literature (more frequently 1.5 or 2), and it changed depending on the treatment, however, due to the method of inoculum preparation, the cell load and activity were always high enough to ensure a prompt substrate degradation, and AD started nearly immediately after the start of incubation (Fig. 2). Conversely, 2 d of lag phase were detected for the AD of SCG by Vitez et al. (2016), whereas Luz et al. (2017) estimated a lag time >9 d for the AD of soluble SCG in co-digestion with cow manure.

The AD of SCG allowed CH₄ production, both in monodigestion and in co-digestion (Fig. 2). In co-digestion, a higher CH₄ production from nearly the same volume of reaction mixture (50 mL in monodigestion, and 53 mL in co-digestion) was possible, because more VS were present in the reactor.

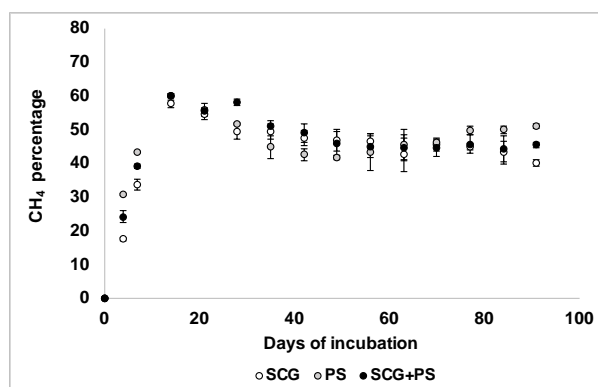


Fig 1. Methane concentration in biogas during anaerobic digestion of spent coffee grounds in monodigestion (SCG) and in co-digestion with pig slurry (SCG+PS). Pig slurry alone (PS) was included as feedstock for comparison. At each measurement time, vertical bars are the standard deviation of the mean

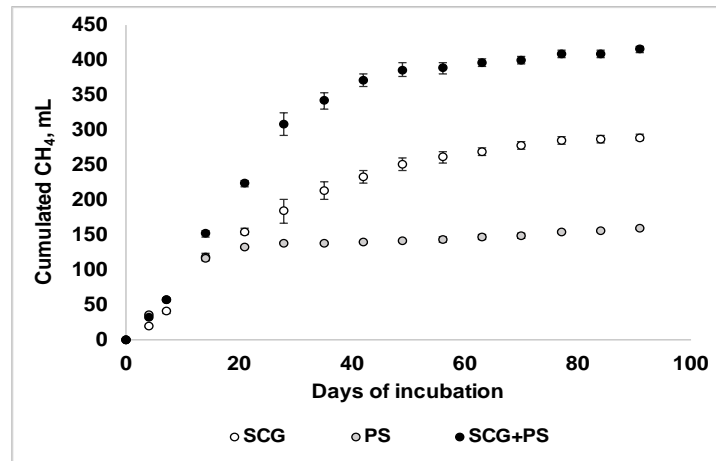


Fig 2. Cumulative CH₄ production during anaerobic digestion of spent coffee grounds in monodigestion (SCG) and in co-digestion with pig slurry (SCG+PS). Pig slurry alone (PS) was included as feedstock for comparison. At each measurement time, vertical bars are the standard deviation of the mean.

Table 2. Volume of CH₄ accumulated 4, 14, and 91 days after the start of the anaerobic digestion, and bimethanation potential (BMP) of spent coffee grounds in monodigestion (SCG) or in co-digestion with pig slurry (SCG+PS). Pig slurry alone (PS) was included for comparison.

Treatment	Cumulated CH ₄ (mL) after						BMP	
	4 d		14 d		91 d (Mmax)		mL CH ₄ g ⁻¹ VS	
SCG	19.4	b	119	b	290	b	290	ab
PS	36.0	a	116	b	159	c	318	a
SCG+PS	31.9	a	152	a	415	a	278	b

3.1. Monodigestion conditions

In our experiment, the initial rate of CH₄ production in SCG (Table 2) was lower than in PS, as only $19.4/1 = 19.4 \text{ mL CH}_{4(\text{STP})} \text{ g}^{-1} \text{ VS}$ were produced 4 d after the start of incubation, much less than in the PS treatment ($36.0/0.5 = 72 \text{ mL CH}_{4(\text{STP})} \text{ g}^{-1} \text{ VS}$). Equally, the CH₄ production rate, as estimated by the measurements 14 d after the start of incubation (Table 2), was the same in the SCG and PS reactors, when the comparison was based on the amount of gas produced per reactor. However, when related to the initial VS content, the amount of CH₄ produced in the SCG reactors in the linear phase of CH₄ production was $119 \text{ mL CH}_{4(\text{STP})} \text{ g}^{-1} \text{ VS}$, that is lower than that calculated for PS ($116/0.5 = 232 \text{ mL CH}_{4(\text{STP})} \text{ g}^{-1} \text{ VS}$). Reasons can be identified with the need for microorganisms to multiply and adapt to an environment richer in VS. Alternatively, an initial inhibitory effect of some SCG component can be considered. Sousa et al. (2015) reported that SCG can have an inhibiting effect towards selected microbial pathogenic species (*S. Aureus*, *E. Coli* and *Candida* sp.) because they are rich in bioactive molecules. However, the amount of gas produced per reactor at the end of AD (91 d after the start of incubation; Table 2) was higher in SCG than in PS, and the BMP of the two feedstocks did not differ significantly.

The BMP value for SCG in monodigestion was on average 290 mL CH_4 (Table 2). This amount is in agreement with those reported by Vitez et al. (2016), who used SCG from a coffee shop; it was slightly lower than that reported by Kim et al. (2017) and

Valero et al. (2016), who worked on SCG coming from a cafeteria, obtaining 314 and $318 \text{ mL CH}_4 \text{ g}^{-1} \text{ VS}$, respectively. The amount of CH₄ that can be produced from SCG, being of the same order of magnitude of that obtainable from other, frequently used, feedstocks, makes it a good feedstock for AD. In fact, a part from PS, which in our experiment allowed a BMP of $318 \text{ mL CH}_4 \text{ g}^{-1} \text{ VS}$ (Table 2), from grass silage it is possible to obtain $320 \text{ mL CH}_4 \text{ g}^{-1} \text{ VS}$ (Luna-delRisco et al., 2011), from wheat straw, $276 \text{ mL CH}_4 \text{ g}^{-1} \text{ VS}$ (Bauer et al., 2010), from maize residues, $317 \text{ mL CH}_4 \text{ g}^{-1} \text{ VS}$, from barley straw, $229 \text{ mL CH}_4 \text{ g}^{-1} \text{ VS}$, and from rice straw, $195 \text{ mL CH}_4 \text{ g}^{-1} \text{ VS}$ (Dinuccion et al., 2010).

3.2. Co-digestion-conditions

In co-digestion, 152 mL of CH₄ per reactor were accumulated 14 days after the start of the incubation in the SCG+PS treatment (Table 2), that is, +29%, on average, in comparison with monodigestion. This amount corresponds to a daily methane production of $10.9 \text{ mL CH}_4 \text{ d}^{-1}$ for SCG+PS treatment, vs 8.5 and $8.3 \text{ mL CH}_4 \text{ d}^{-1}$ for SCG and PS, respectively. Thus, co-digestion can increase the daily rate of CH₄ production.

Equally, the co-digestion with PS allowed an increase (+85%, on average) in the total amount of CH₄ produced per reactor at the end of the AD period (Table 2), in comparison with monodigestion treatments. In fact, 415 mL CH_4 (1.5 g VS) could be obtained per reactor in co-digestion, instead of 290 mL CH_4 in SCG (1 g SV) and 159 mL in PS (0.5 g VS),

compared to an increase of only 6% in the volume of the feedstock.

The theoretical BMP expected from co-digestion is: $290 \times 0.67 + 318 \times 0.33 = 299 \text{ mL CH}_4$, when considering the VS contribution of SCG to the VS weight unit ($1/1.5 = 0.67$), and that of PS ($0.5/1.5 = 0.33$). This theoretical BMP is not significantly different from that measured in co-digestion, on the basis of a t-test.

The type of substrate selected for co-digestion of SCG can significantly influence the fate of the AD process. Kim et al. (2017) evaluated several substrates in co-digestion with SCG, and found differing trends depending on the substrate type. When SCG were digested with differing percentages of cheese whey, the BMP was never affected by the recipe, whereas when they were co-digested with food waste the BMP decreased for increasing percentages of SCG. The SCG inclusion in the recipe with *Ulva* (marine macroalgae) or waste activated sludge remarkably increased the BMP of AD. Qiao et al. (2013), working in thermophilic conditions, noticed a positive effect when SCG was in co-digestion with waste activated sludge. As no difference between theoretical and actual BMP could be detected, our results demonstrate that, in this experiment, a negative as well as a positive interaction between co-ingredients can be excluded.

3.3. Relationship between SCG composition and anaerobic digestion

Spent coffee grounds are rich in caffeine and polyphenols (Tab.1). These compounds have a recognized antimicrobial activity (Daglia, 2012; Nonthakaew et al., 2015). Caffeine can easily penetrate the bacterial cell walls and inhibit DNA synthesis, in so interfering with bacterial growth (Sundarraj and Dhala, 1965). Polyphenols are known to affect microbial growth by acting on enzyme activity or on signal transduction pathways to cell receptors (Daglia, 2012; Field et al., 1989). Several authors have reported polyphenol toxicity on methanogens (Field and Lettinga, 1987; Kayembe, 2013), to the point of proposing polyphenols as an ingredient in the diet of ruminants to reduce the emissions of CH_4 from the rumen of these animals (Patra and Saxena, 2010). Battista et al. (2014) report that the inhibiting action of polyphenols against methanogens increases for increasing amounts of polyphenols in the waste derived from olive oil production. On the contrary, no evidence exists on the inhibition of methanogenesis by caffeine. In fact, some authors suggest the possibility to produce CH_4 from caffeine, although in specific digestion conditions (Chen et al., 2018; Prabhudessai et al., 2009).

In our experiment, the presence of inhibitory compounds in the SCG reactors affected the digestion performances at the start and in the linear phase of CH_4 production. The lower, even though not significantly different, BMP in the SCG than in the PS treatment confirmed the initial trend. This is due to the intrinsic characteristics of the feedstock in the recipe. Spent

coffee grounds have shown a different behaviour, in comparison with PS, due to their different composition. However, in the end, the BMP of SCG+PS was not significantly different from the theoretical one. Therefore, in co-digestion, the lower AD performances of SCG were compensated by the contribution of PS to CH_4 production, and the co-digestion solution was the best, both on an absolute basis (volume of CH_4 produced per reactor) and in relation to the VS content of the feedstock.

It remains to be ascertained which component in the SCG feedstock had a delaying effect on the start of AD.

Marchetti et al. (2016), studying the effect of selected feedstock components on CH_4 production from wetland biomass, did not find any negative correlation between polyphenols, in concentrations similar to those in this paper ($15 \text{ mg tannic acid g}^{-1}$ dry matter, on average) and CH_4 production.

Among SCG components, lipids represent an important fraction ($>9\%$, Table 1). Lipids can interfere with anaerobic digestion of SCG (Qiao et al. 2013) due to the release of long-chain fatty acids (LCFA) in consequence of triglyceride hydrolysis. A negative effect of LCFA on AD has been recognized on the acetogenic activity of syntrophic bacteria (Alves et al., 2009). However, Valero et al. (2016) did not find any limiting effect of lipids on the anaerobic digestion of SCG.

Based on data of Table 1, 16.8% of SGC TS was in the ADL fiber fraction, mainly containing lignin. Lignin content in feedstock has been repeatedly and negatively correlated to CH_4 production (El Achkar et al., 2016; Marchetti et al., 2016). Our samples contained 19% cellulose, as estimated by subtracting the ADL to the ADF fiber fraction (Table 2). The presence of lignin impedes the cellulose utilization, because cellulose, when linked to lignin, becomes completely recalcitrant (Jimenez et al., 1990). Based on the amount of biogas produced per reactor, our results are in agreement with those of Vitez et al. (2016), who observed no inhibition for AD due to the presence of materials such as caffeine, tannins, and polyphenols, whereas different conclusions can be drawn on the basis of biogas production per VS unit.

4. Conclusions

The biological methane production from spent coffee grounds can represent an interesting alternative to landfill disposal. The biomethanation potential of this feedstock amounts indeed to $290 \text{ mL CH}_4 \text{ g}^{-1} \text{ VS}$, which is comparable to that of other substrates currently used as ingredients in anaerobic digestion, even though lower than BMP of pig slurry ($318 \text{ mL CH}_{4(\text{STP})} \text{ g}^{-1} \text{ VS}$). Co-digestion of spent coffee grounds with pig slurry did not seem to involve any inhibition effect, as in this case BMP ($278 \text{ mL CH}_{4(\text{STP})} \text{ g}^{-1} \text{ VS}$) was not statistically different from the theoretical one.

Our in-batch results suggest the possibility on the one hand of increasing biogas yields from pig

slurry using spent coffee grounds as co-substrates, while keeping nearly constant the reactor volume. On the other hand, pig slurry could be conveniently utilized as hydration medium, as requested by the low moisture content of spent coffee grounds, that need resuspension when digested in wet anaerobic digestion processes. Clearly, the hypothesis of using pig slurry in co-digestion with spent coffee grounds needs to be substantiated by in-continuous experiments, specifically devoted to the study of chemical and physical interaction effects between feedstock ingredients on the parameters more appropriately describing the performances of the anaerobic digesters.

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