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Batch and continuous biogas production arising from feed varying in rice straw volumes following pre-treatment with extrusion

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Abstract

This paper studies the synergistic effects on biogas production obtained when different feedstocks are co-digested with varying proportions of rice straw and explores their behavior at the laboratory scale in continuously stirred digesters. Evaluative measures included methane production, volatile solids degradation, ash accumulation, and extrusion effectiveness. The effect of extrusion on the production of energy was also investigated. Results indicated that continuous stirred digesters fed with substrates composed of 10% or 30% of ensiled rice straw (on total FM) produced 146.1 and 140.0 l_N CH_4 $kgDM^{-1}$ day^{-1}, respectively. When extrusion was employed, organic matter degradation was promoted and methane production was significantly raised—by as much as 16%. For the feeds containing 10% rice straw, the increase in obtained energy was higher than the energy needed for the extrusion, but the energy balance was close to zero when the percentage of rice straw was the 30% of the feed.

Keywords: rice straw, biogas, extrusion, energy balance, co-digestion, continuous stirred digester

1. Introduction

One potential solution to the food—feed—fuel debate is to use agricultural residues, as opposed to energy crops in anaerobic digestion plants. Among the various residue alternatives, rice straw is one of the most abundant and renewable energy sources in the world. Globally, 2012 production was about 720 million tons, of which 4 million was derived from Europe (Food and United Nations, 2014). Italy is the largest producer of rice in Europe and represents 40% of the continental crop production. Concentrated in northwestern Italy, more than 1.5 million tons of rice are harvested annually from a surface area of 246,500 ha (Food and United Nations, 2014). The dry weight ratio of rice straw to rice grains with chaff (between 0.8 and 1.2 (Zhang et al., 2012)) makes it possible to estimate that the rice crop of northwestern Italy alone produces 1.2-1.8 million tons of rice straw annually that require management.

Soil incorporation often fails as the optimal way to manage rice straw residue. Indeed, available research suggests that the practice can reduce crop yields by increasing foliar disease and degrading soil conditions (Zhang and Zhang, 1999). Moreover, not all soil conditions permit effective rice straw degradation, a process critical to preservation of ideal organic matter conversion for good soil fertility (Devèvre and Horwáth, 2000). In adverse pedo-climatic conditions in which organic matter degradation is slowed, some fermentative processes and toxic substance productions may compromise rice yields. For all these reasons, farmers often dispose of rice straw improperly, which can indirectly lead to widespread environmental concerns. Open-field burning is one such disposal method; however, the practice is now avoided in several Italian areas because of its polluting effects. Consequently, rice straw field removal often emerges as the best alternative (Bird et al., 2002; Hill et al., 2006).

One promising alternative to rice straw disposal problems in concentrated rice production regions is enhanced anaerobic digestion of the biomass. In fact, the energy content of rice straw (6,533 kJ kg^{-1}) warrants consideration of this residue as a renewable resource for energy generation (Zhang and Zhang, 1999). Several rice straw compositional constraints must first be overcome to make its digestion feasible: low available structural carbohydrates (leads to an inadequate supply of net energy supply); silicified surface layer, lignin, and associated phenolics; intrinsic cell wall carbohydrate properties, such as crystallinity and esterified group substitution on a xylan backbone (Taherzadeh and Karimi, 2008). To mitigate the limited digestibility that each of these impediments presents in anaerobic digestion, the residue must be well managed with a pretreatment to reduce particle size and enhance degradability.

In general, rice straw is not considered a high methane-producing biomass due to its high ash content (15-20%) and the low digestibility of its fibers. Previous studies have measured rice straw methane production in a range between 110 and 180 l kg^{-1} of dry matter (Chen et al., 2014; Menardo et al., 2012); however, these analyses were limited to dry rice straw produced by the most common method of residue conservation. In fact, rice straw that is to be used as anaerobic digestion plant (ADP) feedstock is preferably ensiled to retain high moisture content while reducing biomass lignification (Ghasemi et al., 2013). Moreover, ensiled rice straw also limits ADP biogas outlet problems associated with crust formation from floating biomass. Finally, the high ash content and low digestibility of rice straw also causes the dry matter inside the digester to increase, which can consequently stress the mixing system and lead to higher energy consumption. To mitigate rice straw flotsam and ease digestate mixing—while simultaneously increasing rice straw digestibility and methane yield—pretreatment is necessary. Mechanical pre-treatments are not only practical at the individual farm scale, but they have demonstrated results at reducing particle size and improving methane production of ligno-cellulosic biomasses (Hjorth et al., 2011; Chen et al., 2014). Among the various mechanical options, extrusion pre-treatment works by shearing, heating, and disrupting the lignocellulose structure of the biomass to shorten and defibrillate the fibers (Kratky and Jirout, 2011).

This study had two main objectives:

- to investigate the effect of synergies on AD biogas production when different feedstocks are co-digested with varying proportions of rice straw in batch;
- to explore the behavior of increasing rice straw amounts at the laboratory scale in continuously stirred digesters (CSD), in terms of methane production, VS degradation, dry matter, ash accumulation, and the effect of feedstock extrusion.

2. Materials and methods

2.1. Biomass sampling and feed composition

Four different feeds of increasing proportions of rice straw were analyzed for methane production in batch trials. Two of these feeds were selected in a follow-on step, according to their methane potential yield, and analyzed in a laboratory scale CSD.

The four initial feeds were composed of three different feedstocks: rice straw silage (RS), maize silage (MS), and triticale silage (TS). The analyzed feeds contained differing amounts of rice straw, 10% (RS10), 30% (RS30), 50% (RS50), and 70% (RS70). The remaining proportion of each feedstock was constituted of maize silage and triticale silage in a 2.5:1 ratio on a fresh weight basis (Table 1).

Feedstock samples were collected at a farm sited in San Germano Vercellese in northwest Italy (45°27’ N lat., 8°26’ E long., 161 m a.s.l.). The biomasses were shredded into1-2 cm particles and then ensiled in large plastic film silos. Core drilled samples of the feedstocks were retrieved three months after storage directly from the inner part of the silos, after which they were placed into vacuum-sealed bags and stored at -18°C until trials began.

2.2. Extrusion pre-treatment

All three feedstock extrusions were performed at an ADP in San Germano Vercellese using a two counter-rotating screw extruder driven by a 74 kW motor, model MSZB-74E, produced by Lehmann Maschinenbaum GmbH, Pöhl, Germany. The expansion/wearing zone beyond the compression zone of the device consisted of double-spaced counter-twisting screw blades. At the outlet of the extruder, an adjustable plate controls the size of the opening, which can be varied in size to modify biomass compression. The plate was adjusted to 70% of its maximum for this experiment.

A total of 200 kg of each biomass (RS, MS, and TS) was fed into the extruder as a test. The first 100 kg were used to cleanse the extruder of the previous pretreated biomass, while the rest represented pure biomass and was sampled at the outlet. A total of 10 kg of each extruded biomass was collected in plastic (polyethylene) containers and stored at 18°C until analysis.

2.3. Biochemical methane potential (BMP) test in batch.

The BMP tests were conducted according to VDI 4630 (2006), in 2.0 l capacity batch digesters at 40°C for 60 days with manual stirring at least once per day. The samples and inoculum were weighed in batches at a ratio of 1:2 (organic dry matter (VS) basis). The volume of produced biogas was monitored by means of a Ritter Drum-type Gas volume meter (TG05/5, Ritter Apparatebau GmbH & Co. KG, Bochum, Germany) every 1-2 day, depending on the biogas produced. Simultaneously, the biogas composition (CH_4, CO_2, H_2, O_2, H_2S concentrations) was determined by means of a gas analyzer with infrared sensors (model XAM 7000, Drägerwerk AG & Co. KgaA, Lübeck, Germany).

Each biomass/feed was digested in triplicate; the control sample was represented by inoculum alone. The inoculum used in the batch trials was the mechanically separated liquid fraction of the digestate produced by the biogas plant in San germane Vercellese (45°27' N lat., 8°26' E long.). The plant is usually fed with maize-, triticale-, or ryegrass-silage and ensiled rice straw, so the contained microbial population needed no adaptation for the tested feedstocks. Characteristics of the inoculum included 6.6% DM content, ash at 30.7% of DM, pH = 7.8, and NH_3-N of 0.23% (on fresh matter (FM)). The biogas volume produced by the inoculum was measured and subtracted from the biogas yield obtained from each sample. The gas production was normalized to 0°C and 101.3 kPa, and expressed as l_N per kg of VS. The batch headspace volumes allowed calculations of CH_4 and CO_2, using a correction factor as reported in VDI 4630 (2006). The VS degraded during the anaerobic digestion (AD) in batch was calculated as the difference between the VS amount of the feedstock and the residual VS contained in the digestates at the end of AD (60 days).

2.4. Continuous anaerobic digestion experiment

After identification of the two feeds with the best methane productions (RS10 and RS30) in the BMP test in batch, the feeds were analyzed and compared in CSD trials. The experiment was carried out at the same temperature as the batch trials (40°C) within a temperature-controlled chamber, using six lab-scale 7.0 l plexiglass CSDs, with 5.5 liter liquid working volumes. Each reactor was equipped with a mixing system composed of a vertical mixer with a geared motor installed on its top. The mixing speed inside the reactors was set at about 4 rpm. The reactors were equipped with an inlet—outlet system for the feed supply and digestate discharge. The inlet system was placed on the side of the reactor at its mid-height to facilitate biomass insertion directly into the digestate and to reduce to a minimum the introduction of air into the inlet of the reactor. The inlet system was sealed with a rubber cap. The outlet system, consisting of an approximately 35 cm long rubber pipe (diameter 6 cm), was situated at the bottom of the reactor for digestate collection. The outlet pipe was sealed by plastic cap and maintained in a vertical position by an elastic band. A pipe situated at the top of the reactor was connected to Tedlar® (DuPont Co., Wilmington, DE, USA) gas bags with Tygon® tubing (Saint-Gobain S.A., Courbevoie, France) to collect the produced biogas (Dinuccio et al., 2013).

The experiment lasted 186 days and was carried out in triplicate. The reactors were started with 100% inoculum from the full scale ADP in San Germano Vercellese and then fed for approximately two months with the two different feeds, until a steady state was achieved. The inoculum used for experimental start contained 5.8% of DM, 20.1% of ash (DM basis), pH 7.8, a C:N ratio of 7.2, and a N-NH_3 percentage of 0.23% (FM basis). On day 60, the experiment was initiated and the reactors were simultaneous fed thrice weekly with the RS10 and RS30 selected feeds for the remainder of the experiment. Feet RS10 was composed of 3.0±0.2 g of RS, 21.7±0.6 g of MS, 8.4±0.2 of TS; feed RS30 was characterized by 9.1±0.5 g of RS, 17.4±0.7 g of MS, and 6.7±0.2 g of TS. Prior to feeding, an amount of digestate equivalent in volume to the inlet was discharged from each CSD. A small quantity of discharged digestate (20.0 g per day) was re-circulated with the feedstock every day, as was a small volume of water (37 g) to maintain a stable DM content inside the digester. The operative CSD parameters for both substrates were an organic loading rate (OLR) of 2.0 kgVS/ m^3 digester per day and a hydraulic retention time (HRT) of 60 days.

After the adjustment period (day 0-60), feeding proceeded for two more months (days 60-120) with ensiled feedstock alone. During the final two experimental months (days 120-186), the ensiled feedstocks were substituted with ensiled plus extruded feedstock to highlight the effect of the extrusion on the methane yield of both analyzed feeds. The OLR and the HRT were maintained for the full experimental period (186 days).

Biogas and CH_4 yields were measured three times each week throughout the experimental period. Biogas was collected in 10-30 liters Tedlar® bags. Biogas volumes and gas compositions were determined through the same methodology and instruments adopted for the batch trials. The recorded data were normalized at standard temperature and pressure according to VDI 4630 (2006).

2.5. Analytical methods

The single biomasses and the four feeds were analyzed for DM content by oven drying at 80°C for 24 h and for ash by ignition to 550°C in a muffle furnace. Elemental composition (C, N, H, O, S) was determined by elemental analyzer CHNS-O EA1110 (Carlo Erba), according to UNI EN 15407:2011 and MIP-011 2008 Rev 1.2 for sulfur. Samples were also analyzed for NDF using heat-stable amylase (A3306, Sigma Chemical Co., St. Louis, MO, USA) as described by Van Soest et al. (1991), and for ADF and ADL as described by Robertson and Van Soest (1981). The ammonia nitrogen ($N\text{-}NH_3$) content and pH were quantified in the water extracts.

A fresh sample of each biomass, before and after extrusion pre-treatment, was extracted using a Stomacher blender (Seward Ltd., Worthing, UK) for 4 min in 0.05 mol l^{-1} H_2SO_4 at an acid/sample material ratio (fresh weight) of 5:1. An aliquot of 40 mL of sample acid extract was centrifuged at 3,622 × *g* for 4 min, and the supernatant was filtered with a 0.20-µm syringe filter and used for quantification of lactic and monocarboxylic acids (acetic, propionic, and butyric acids) with an HPLC (Agilent Technologies, Santa Clara, CA) (Canale et al., 1984). Ethanol was determined by HPLC, coupled to a refractive index detector, on a Aminex HPX-87H column (Bio-Rad Laboratories, Richmond, CA). The analyses were performed isocratically under the following conditions: mobile phase 0.0025M H_2SO_4, flow rate 0.5 ml/min, column temperature 37°C, and injection volume 100 µl (Borreani et al., 2014).

In order to analyze the AD process of the two feeds (RS10 and RS30), the digestate for each replicate was sampled about every 10 days and analyzed for the following parameters: DM, ash, organic carbon, Kjeldahl nitrogen, pH, N-NH_3 and acetic, propionic and butyric acid. For VFAs determination, the digestate samples were extracted using a Stomacher blender for 4 min in 0.05 mol l^{-1} H_2SO_4 at an acid/sample material ratio (fresh weight) of 1:1.

2.6. Data analysis

The statistical analysis was performed using SPSS Version 17. The data were analyzed by one-way ANOVA and by Tukey's test ($P < 0.05$) for post hoc comparison. Levene´s test was previously used to assess the homogeneity of the sample variances.

The electric energy demand necessary to extrude RS10 and RS30 was calculated (kWh_{el} t^{-1} of FM) using the data recorded during extrusion and the main technical parameters of the extruder used for pre-treatment, by the equation (1):

$$E_{extrusion} = (V \cdot I \cdot cos\varphi \cdot \sqrt{3}) / (C \cdot 1000) \qquad (1)$$

where $V = 400\mathrm{V}$, $cos\varphi = 0.8$, I was the electrical intensity, which ranged between 94.5 A (for RS10) and 115.9 A (for RS30). C was the extruder working capacity and was 5.2 t/h for both feeds. Electrical energy produced by untreated and pretreated biomasses, expressed as $\mathrm{kWh_{el}\ t^{-1}}$ of FM, was calculated using equation (2):

$$\mathrm{E_{sample}} = (Y \cdot HHV \cdot \eta_{el}) / 3.6 \qquad (2)$$

where Y was the specific methane yield of untreated and treated samples, expressed in $\mathrm{m^3}$ per tonnes of FM, HHV (Higher Heat Value) was 39.79 MJ $\mathrm{N^{-1}m^{-3}}$ and η_{el} was the co-generator (CHP) electrical efficiency estimated as 40%. The methane yield of the untreated biomasses was deducted from the yield of each pretreated biomass to obtain the real methane yield increasing value.

3. Results and discussion

3.1. Chemical characteristics of the biomasses

The DM content of the ensiled biomasses ranged between 34.2 and 36.7% (Table 2), which were adequate for ensiling conservation (Heiermann et al., 2009). Lower moisture values may prevent fermentation start, whereas higher moisture values may promote mold development in the silage (Heiermann et al., 2009). The DM of RS was adequate at 34.2% because it skipped the field drying phase and was ensiled immediately after harvest.

The ash content was 5.1% for MS and 4.6% for TS, as opposed to the higher 15.1% value for RS due to its high content of siliceous compounds, which are the structural elements in diatoms and a cell wall component in all rice by-products. Siliceous compounds represent about 78-80% of the ash in rice straw (El-Sayed and El-Samni, 2006; Elwan et al., 2006). Studies have shown that silica in rice is not only responsible for fungal disease resistance, but also has a role in several major processes: carbohydrate synthesis, grain yield determination, phenolic synthesis, and plant cell wall protection (Mengel and Kirkby, 2001). Even though these compounds are fundamental for rice growth, they make rice by-products less suitable for biogas production because they are inert and inedible to the microorganisms that produce biogas. In RS, the organic compounds represented just 84.9% of DM, whereas in MS and TS, they comprised 94.1% and 95.4%, respectively.

Feedstock pH was in the optimal range for ensiling and for rice straw (4.1). The rice straw ensiling process proceeded well as was confirmed by its observed high quality at sample collection. Despite the far lower sugar content of rice straw *versus* maize or ryegrass, ensiling can still optimally preserve the biomass, if ensiled immediately after harvest when the moisture is about 65% (Shinozaki and Kitamoto, 2011). Ensiling is a well-known procedure for preserving forage crops with minimal nutrient loss. Various authors have reported this preservation method as also very suitable for biogas production (Shinozaki and Kitamoto, 2011; McDonald et al., 1991).

The elemental composition of RS was found to have low carbon content (42.7%) because of its high ash amount, and lower hydrogen and oxygen amounts compared to the two other feedstocks. The oxygen in RS refers to the organic fraction, while the amount connected to silicate is included in the ash content and was not characterized. MS contained higher amounts of nitrogen and sulfur than TS and RS due to its abundance in proteins, but it had a very low C/N ratio (42.2).

Fibers composed rather similar proportions in the rice straw and triticale silage, whereas MS contained lower values for both CEL (16.4%) and H-CEL (14.6%). Rice straw contained a typically low amount of lignin (3.1%) compared to the other cereal biomasses, which Van Soest (2006) also observed relative to other cereals.

Chemical analysis of the four feeds demonstrated that it is possible to smooth the effects of some problematic parameters by mixing biomasses. The high ash content of rice straw was diluted in RS10 and RS30, in particular. Similarly, the C/N ratio was reduced when nitrogen-rich maize silage was added (Li et al., 2013).

The acids and ethanol concentrations of the ensiled feedstock, before and after extrusion, are shown in Table 3. The main fermentation products for all three biomasses were acetic and lactic acids, whereas butyric and propionic acid were below detection limits (< 0.1 g kg $^{-1}$ DM) in all silages. The values are consistent with values reported in previous studies for biomasses ensiled for more than three months (Borreani et al., 2014; Fang et al., 2012; Cerda et al., 2003). VFA concentrations in rice straw, together with low pH values, confirmed that fermentation proceeded correctly. The VFA and ethanol concentrations in the samples did not show an effect from extrusion, according to the short process duration and relatively low temperature reached during pre-treatment. Moreover, the samples were collected in a closed system immediately after the extruded biomass outlet.

3.2. Biogas and methane yields from single feedstock and mixed feedstocks

Table 4 displays the results of the BMP test for the separate production of biogas and methane of the feedstocks. Rice straw produced 556.5 l_N kgVS^{-1} of biogas containing 50.3% of methane. Previous studies (Ye et al., 2013; Chandra et al., 2012) have generally reported lower biogas yields for rice straw (180-220 l_N kgVS^{-1}), but from dried rather than ensiled rice straw samples. Dry conservation, commonly used for an extended period of preservation, is inadequate for preservation of feedstock for biogas production. Dry conservation results in increased ligno-cellulosic compound recalcitrance that reduces cellulose availability for microorganism degradation. Some experiments that analyzed biogas production from ensiled rice straw revealed that it is possible to obtain higher methane yields from ensiling—instead of drying and baling—feedstocks (Gu et al., 2014; Chen et al., 2014). The methane yields obtained by these researchers confirmed the results observed in this study.

Maize and triticale biogas productions were 688.3 l_N $kgVS^{-1}$ and 612.4 l_N $kgVS^{-1}$, respectively. Triticale silage contained more methane (52.4%), but its degradation was lower compared to the other analyzed biomasses, at just 58.3%. Considering that TS fibres composition is so similar to RS, the lower VS degradability of TS may relate to its fibre structural characteristics.

In tandem to the individual BMP analysis, we tested the methane production from four different feeds of increasing rice straw amounts to evaluate the effect of rice straw in a feed and the possible synergistic and/or inhibitory effects on methane production. The biogas and methane production, and the calculated VS degradation for each feedstock (RS10, RS30, RS50, and RS70), were determined and are displayed in Figure 1.

At first, evidence showed that as rice straw increased in the feed, the biogas and methane yield decreased significantly ($P < 0.05$). In fact, the biogas yield decreased from 652.6 l_N $kgVS^{-1}$ in RS10 to 539.6 l_N $kgVS^{-1}$ in RS70. Methane showed the same trend. While significant differences had already been observed between RS10 and RS30 both for biogas and methane production, the VS degradation of these low rice straw content feeds behaved very similarly. Specifically, RS10 yielded 347.8 l_N $kgVS^{-1}$ of methane and when the rice straw percentage rose to 30% (RS30), the methane yield decreased 8.8%. The decrease then extended when the percentage reached 50% (RS50), such that a gap of as much as 17% existed for the methane production between RS10 and RS50. An increase in the proportion of rice straw in the feed between 50% and 70% showed no further effect on methane yield and it remained stable at about 286-287 l_N $kgVS^{-1}$. Stated another way, between RS10 and RS50, an increased rice straw feed determined a significant ($P < 0.05$) reduction in methane production; when rice straw content exceeded 50%, the effect was null.

Reduced methane yields were not solely attributable to rice straw increases in the feed. The relative decrease in maize silage, as rice straw rose, also affected yield. Over the four feeds, maize fell from its highest (64%) in RS10 to its lowest (21%) in RS70. Triticale silage also decreased in amount as rice straw increased, but the reduction was less marked. Triticale varied from 26% of RS10 to 9% of RS70.

The BMP test results on the four feeds were as expected for the individual methane productions from the feedstocks. When calculations using BMP values were performed to estimate potential feed productions, some synergies and inhibitory effects were revealed. For RS10, the potential methane production was lower compared to that obtained by BMP tests. Indeed, the BMP test showed a production 4.5% higher than the potential one ($P < 0.05$). This means that mixing biomasses of various chemical characteristics can improve digestion and anaerobic fermentation inside the digester. Anaerobic digestion of a lignocellulosic-only biomass would eventually lead to nitrogen and buffer capacity depletion, which would inhibit the digestive process (Li et al., 2013). Therefore, for a substrate of this type, co-digestion is the recommended option, such as a rice straw coupled with a feedstock of higher nitrogen content. Of the several experiments performed mixing rice straw and different manure types (poultry, swine, or cattle manure) or organic waste (Li et al., 2013), little has been written on the synergies among different solid feedstocks.

The C/N ratio varied across the various feedstocks: rice straw: 57.7, triticale silage: 60.4, and maize silage: 42.2. This suggests that the slightly-balanced nutrient composition in the RS10 feed supported methane production and stabilized the process. For RS30 no synergistic or inhibitory effects were observed, whereas significant ($P < 0.05$) inhibitory effects were obtained for both RS50 and RS70. Observed productions were lower than calculated productions by 7.5% and 13.5%, respectively. A high rice straw amount in a biogas feed depressed methane production due to the lower digestibility of rice straw and its high ash content, relative to maize silage. It may also have inhibited the fermentation process. The VS degradation was 64-65% both for RS10 and RS30. As rice straw increased as a percentage of the feed, the VS degradation significantly ($P < 0.05$) decreased, by as much as 53%.

3.3. CSD trials: characterization of digestate and the effect of feedstock extrusion.

The feeds that produced the most methane in the batch experiment, RS10 and RS30, were used as feedstock in continuous mixing digesters (CSTR) for two determinations: to observe the long-term effect of rice straw in a pilot digester and to evaluate the effect of feedstock extrusion. Both feeds maintained the same HRT (60 days) and OLR (2.0 kgVS m^{-3}) throughout the experiment.

The daily methane productions (once production stabilized) were similar for both feeds, at 1.5 l_N day^{-1} (Table 5). Similarly, specific yields, expressed on DM or VS content, showed no significant differences between the two feeds, as RS10 produced 146.1 l_N $kgVS^{-1}$ per day and RS30 produced 140.0 l_N $kgVS^{-1}$ per day. The variation was not statistically significant, in contrast to the results observed during in the batch trials. One explanation is that the everyday uploading of fresh biomasses smoothed any differences that occurred under the stable conditions of batch trials. Figure 2a reports the daily methane productions, expressed on kg of added fresh biomass. RS10 showed a slightly higher yield compared to RS30, but overall, feed behaviors were quite comparable. When feeding was substituted with extruded biomasses, methane yield and digestate chemical variation occurred quickly in all digesters. The methane production increased significantly ($P < 0.05$) after a few days and then immediately stabilized at higher levels in both feeds. In the case of RS10, the mean methane specific yield increased about 15.7%, whereas the RS30 increase was less pronounced (10.6%), likely due to the high amount of a recalcitrant biomass, such as rice straw.

Dry matter degraded more in RS10 (63.5%) than in RS30 (62.3%) due to the higher ash content in the proportionally higher rice straw feed (Figure 2b). However, this difference was modulated when VS degradation was considered; specifically, RS10 was degraded 67.8% and RS30 was degraded 66.7% when VS was added with the feedstock. These values are rather high for agricultural feedstocks that are limited by their ligno-cellulosic compounds. However, the values are similar to work by Gonzalez-Gonzalez and Cuadros (2013) who observed degradation rates between 63.5 and 75.3% of the initial organic matter. While they also performed their work in continuous digestion reactors, they utilized organic waste, which is generally considered more easily degraded than agricultural biomasses.

Nevertheless, the biomasses used in this study were properly ensiled and shredded to a particle size of 1-2 cm—two measures that improve degradation outcomes. Ensiling is reported to preserve biomass, and to increase both biogas production and VS degradation (Liu et al., 2014). Biomass particle size reduction is similarly is well understood to be an effective pre-treatment for biogas production (Menardo et al., 2012). Furthermore, the lack of volatile fatty acids (VFAs) in the digesters indicated that readily degradable organic matter was constantly consumed, caused no overload, and left only recalcitrant solids as the main accumulated solid (Estevez et al., 2012). Biomass extrusion promoted VS degradation (Hjorth et al., 2011) through fibre breakdown and particle size reduction of seeds and large chop, which significantly increased methane production by up to 16%.

As reported in previous studies (Menardo et al., 2013; Hjorth et al., 2011), the effect of extrusion is very effective for maize silage, but less so in biomasses with long, hard-to-degrade fibres, like rice straw. As would be expected with the improved methane yield, extrusion caused enhanced VS degradation. In fact, VS degradation increased 13.1% in RS10 and 14.7% in RS30, with the improvement observed just a few days after we fed extruded feedstock to the digesters.

The digestate collected from the digesters was regularly analyzed for pH, a parameter strictly related to the fermentation trend. The two feeds had a very similar mean pH that held at 7.44-7.45 (Figure 3) during the period when no extruded biomasses were used. Fraction recirculation allowed the pH to remain quite high. However, within a few days after the extruded biomasses were introduced, a slight acidification of the digestates took place. We observed pH fall to 7.33 for RS10 and to 7.39 for RS30, which made the biomasses more digestible and hydrolysable by digestate microorganisms. Hydrolysis was stronger in RS10 as evidenced by the lower pH as compared to RS30.

Contrary to similar pH values, the digestate ash content in the feeds differed significantly from the start of the steady phase. As expected, the higher rice straw percentage in RS30 *versus* RS10 related to a larger digestate ash accumulation. Over time, the ash content difference grew, and despite nearly identical OMD in the two feeds, the inert material accumulation was more even in RS30. As extruded biomasses were introduced, this gap magnified such that after 186 days, ash accumulations in the digestate reached 31% and more than 36% of DM in RS10 and RS30, respectively.

The analysis of fibers in the feedstock and digestate collected routinely after digester feeding allowed determination of the mean fibers degradation, and enhanced observation of the effect of extrusion on their degradation. The H-CEL degradation increased from 82.6% to 88.7% in RS10 and from 82.0% to 88.3% in RS30. In addition to hemicellulose, cellulose was another fiber for which extrusion significantly improved degradation. For both RS10 and RS30 feeds, cellulose degradation increased as much as 9.0%. The hemicellulose and cellulose efficiency increase agreed with results obtained in previous studies (Hjorth et., 2011; Menardo et al., 2013; Chen et al., 2014). As Chen et al. (2014) details, extrusion changes the physical properties (particle size distribution, water retention capacity, specific porosity, specific surface area, and more) and modifies the complex structure of fibers, accelerating H-CEL and CEL degradation efficiency.

As expected, no effect was observed on lignin degradation after biomass extrusion. Indeed, while extrusion is unable to degrade lignin, it can weaken lignin and cellulose bonds and make the cellulose more available to the microorganisms.

The energetic efficiency of extrusion was evaluated by a simple energy balance calculation for both feeds. The energy necessary for biomass extrusion was compared to the energy increase obtainable through pre-treatment. The energy required to pretreat RS10 was 10.1 kWh_{el} per ton of fresh matter and 12.4 kWh_{el} per ton of fresh matter for RS30. The higher amount of rice straw in RS30 caused more consistent energy consumption during its pre-treatment. The high ash proportion and resistance to breakdown of the rice straw increased the consumption of electricity by the extruder during pre-treatment (Menardo et al., 2013). The energy increase obtained during biomass anaerobic digestion, less the energy used for extrusion, resulted in a positive energy balance for RS10. In particular, the energy increase obtained was 40.2 kWh_{el} per tonne of fresh matter, or about three times more than the energy used for pre-treatment. On the contrary, the energy balance was close to zero for the RS30 feed. The energy increase obtained from the extrusion was just 13.4 kWh_{el} per tonne of fresh matter, which was not significantly higher than the energy needed for extrusion. The energy balance made evident that extrusion is a good option to improve anaerobic digestion and biomass methane production; however, when recalcitrant biomasses are a high proportion of the feedstock, the energy needed for pre-treatment limits the increase in produced energy.

4. Conclusions

Ensiled rice straw can be used in ADPs in low percentages to improve the feed C/N ratio, but high amounts can depress the methane production due to its low digestibility and high ash content. ADP feedstock pre-treatment by extrusion prior to entry into the digester is a valid method to improve digestate mixing within the digester and biogas production as well, especially when biomasses of low recalcitrance are used. When the biomass is particularly high in difficult-to-degrade fibers, the energy consumed during extrusion may more than offset the energy produced.

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Figure Captions

Figure 1. Biogas, methane production, and VS degradation of the four feedstocks analyzed in batch. Bars denote standard deviations. The letters indicate significant differences among feed parameters with significance level $P < 0.05$.

Figure 2. Evolution of a) methane production, expressed as l_N kg^{-1} of fresh added biomass, and b) DM degradation, expressed as percentage from the two rice straw feeds before and after extrusion pre-treatment in CSD trials. Results are displayed from day 60, after system stabilization. The vertical line indicates the point at which the extruded feedstock was introduced. Bars denote standard deviations.

Figure 3. Trend of pH and ash content of the CSD digestate collected throughout the experiment that compared two rice straw feeds, before and after the extrusion pre-treatment. Results are displayed from day 60, after system stabilization. The vertical line indicates the point at which the extruded feedstock was introduced. Bars denote standard deviations.

Figure 1.

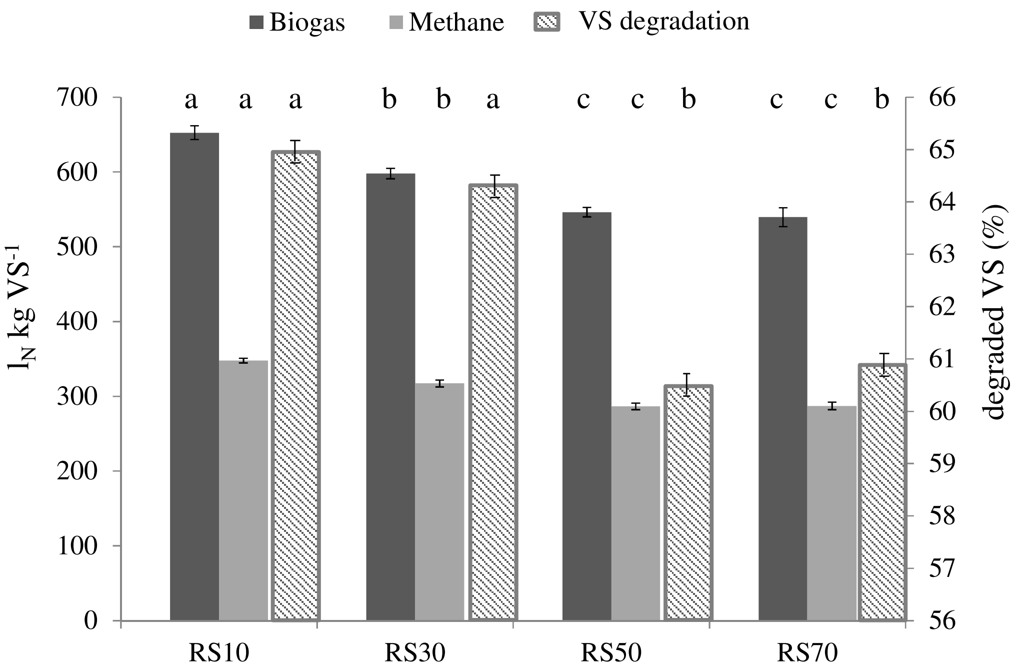
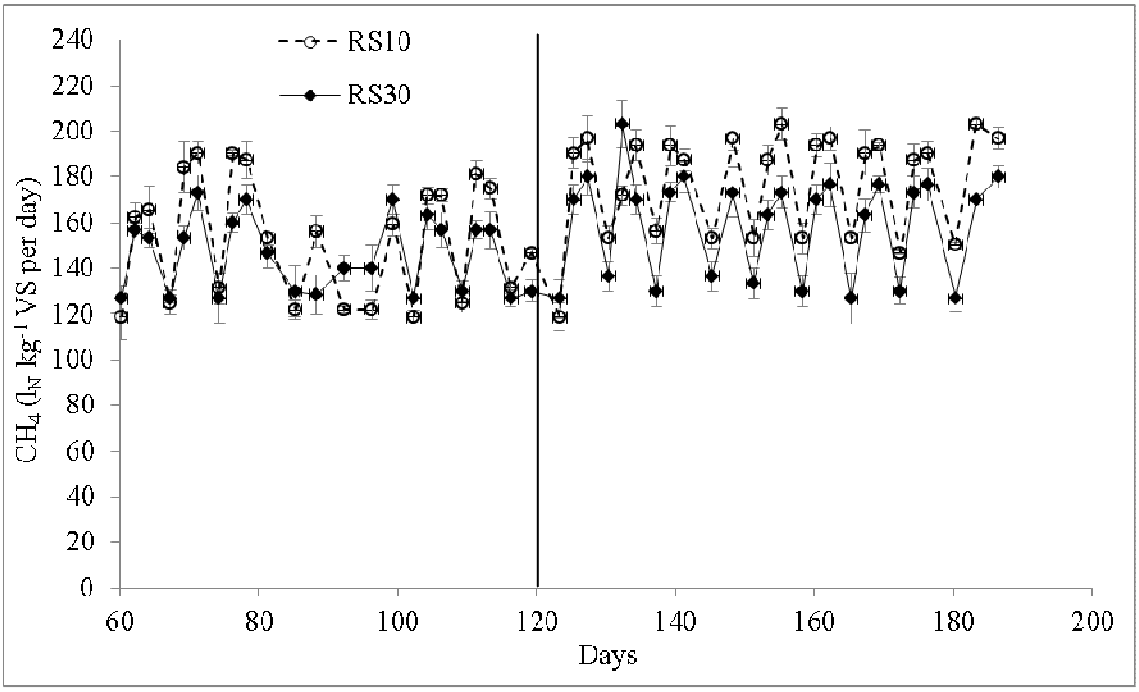

Biogas
Methane
VS degradation
l_N kg VS^{-1}
degraded VS (%)
700
600
500
400
300
200
100
0
66
65
64
63
62
61
60
59
58
57
56
a a a
b b a
c c b
c c b
RS10
RS30
RS50
RS70

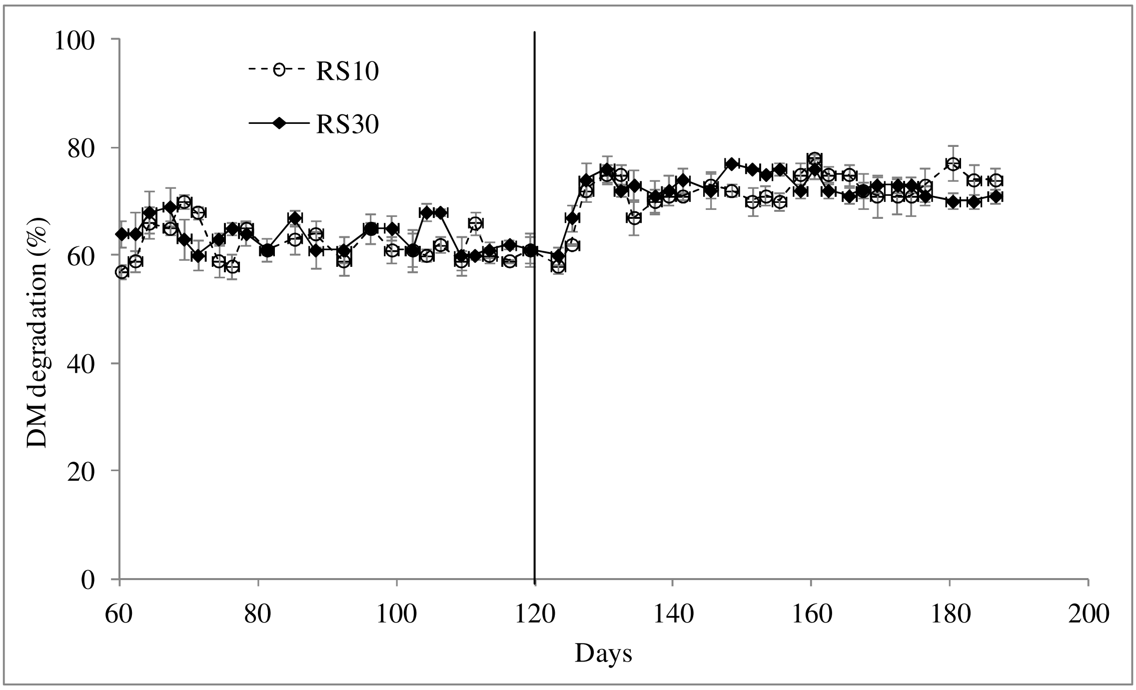
Figure 2.

a)



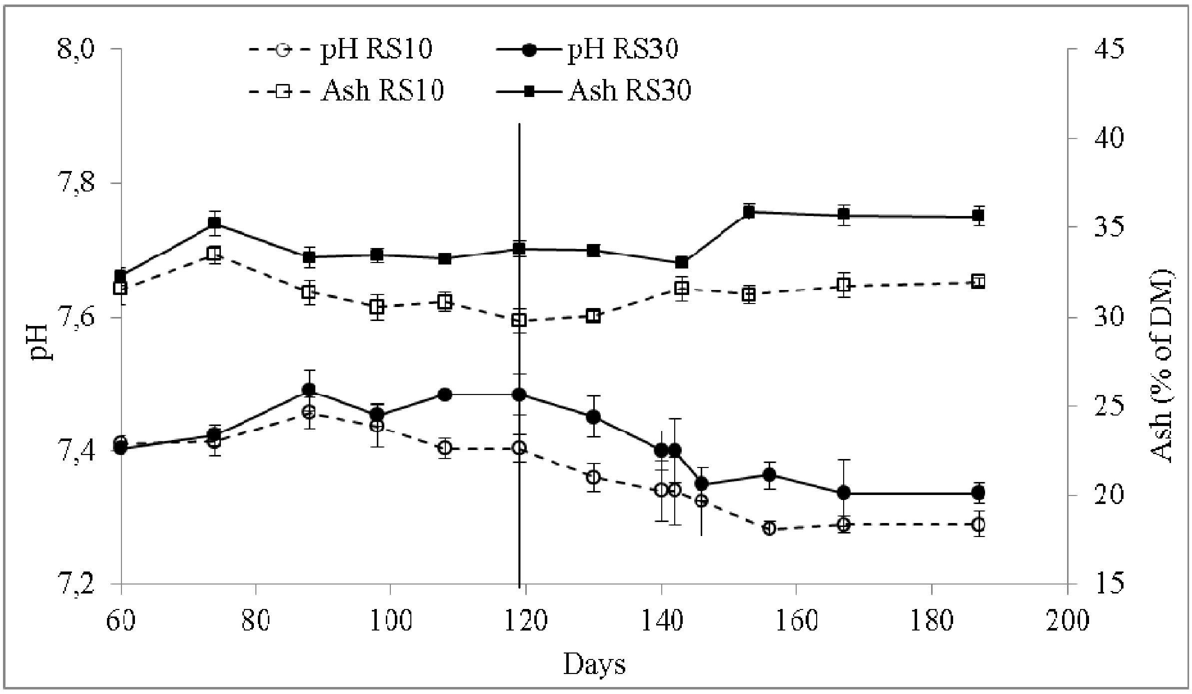
RS10
RS30
CH_4 (l_N kg^{-1} VS per day)
0
20
40
60
80
100
120
140
160
180
200
220
240
60
80
100
120
140
160
180
200
Days

b)



RS10
RS30
DM degradation (%)
0
20
40
60
80
100
60
80
100
120
140
160
180
200
Days

Figure 3.



pH RS10
pH RS30
Ash RS10
Ash RS30
8,0
7,8
7,6
7,4
7,2
pH
45
40
35
30
25
20
15
Ash (% of DM)
60
80
100
120
140
160
180
200
Days

Table 1. Composition of the four different feedstocks analyzed for methane potential in batch. The percentage is expressed on wet weight.

Feeds	RS	MS	TS
		[%]	
RS10	10	64	26
RS30	30	50	20
RS50	50	36	14
RS70	70	21	9

Table 2. Chemical parameters of analyzed biomasses and feeds.

Feedstock	DM	Ash	pH	C	N	H	O	S	C/N	H-CEL	CEL	ADL	N-NH$_3$
	[%]	[% DM]								[% DM]			[% FM]
RS	34.2 (1.5)	15.1 (0.3)	4.1	42.7 (1.2)	0.74 (0.02)	4.2 (0.3)	37.2 (2.1)	0.08 (0.05)	57.7 (1.1)	25.5 (3.5)	25.8 (3.6)	3.1 (0.6)	0.03 (0.01)
MS	36.7 (1.3)	5.1 (0.1)	3.7	46.8 (2.3)	1.11 (0.09)	6.0 (0.1)	40.9 (1.2)	0.13 (0.02)	42.2 (2.3)	16.4 (2.3)	14.6 (2.6)	4.4 (0.6)	0.05 (0.02)
TS	34.3 (0.6)	4.6 (0.1)	4.0	47.7 (0.9)	0.79 (0.02)	6.4 (0.3)	40.5 (1.8)	0.08 (0.01)	60.4 (0.8)	21.6 (1.9)	23.6 (2.6)	4.2 (0.5)	0.04 (0.00)
Feeds													
RS10	35.5 (1.1)	5.9 (0.1)	3.8	46.7 (2.1)	0.98 (0.3)	6.0 (0.1)	40.45 (1.7)	0.11 (0.01)	47.7 (1.8)	19.0 (2.5)	18.3 (2.6)	4.2 (0.7)	0.05 (0.00)
RS30	35.5 (0.9)	8.3 (0.3)	3.8	45.8 (1.5)	0.94 (0.4)	5.53 (0.1)	38.68 (2.1)	0.11 (0.02)	48.7 (1.9)	20.3 (2.0)	18.9 (2.7)	4.2 (0.6)	0.04 (0.03)
RS50	35.1 (1.3)	9.8 (0.1)	3.9	44.9 (1.5)	0.88 (0.7)	5.02 (0.2)	39.05 (1.3)	0.10 (0.01)	51.0 (1.2)	20.6 (2.0)	21.3 (3.0)	3.6 (0.3)	0.04 (0.00)
RS70	34.8 (1.3)	12.1 (0.1)	3.9	44.1 (0.9)	0.82 (0.1)	4.98 (0.1)	39.25 (2.0)	0.09 (0.01)	53.8 (1.3)	24.1 (2.6)	24.3 (2.9)	3.5 (0.1)	0.04 (0.01)

The value in the round brackets is standard deviation (SE).

Table 3. Concentration of volatile fatty acids of the feedstock used in the continuous trials before and after extrusion pre-treatment

	Feedstock	HAC	PRO	LACT	ETH	n-BUT
		[mg kg^{-1}]				
Before extrusion	RS	9.6 (1.2)	<0.1	22.6 (1.1)	8.6 (0.6)	0.9 (0.0)
	MS	33.1 (2.3)	<0.1	51.7 (2.6)	13.7 (0.2)	<0.1
	TA	20.2 (2.3)	<0.1	36.2 (1.6)	16.2 (0.5)	<0.1
After extrusion	RS	8.8 (0.9)	<0.1	24.9 (0.9)	11.1 (1.1)	1.3 (0.1)
	MS	31.6 (2.5)	<0.1	54.3 (2.1)	13.5 (0.9)	<0.1
	TA	18.6 (2.3)	<0.1	36.2 (2.3)	20.5 (0.9)	<0.1

HAC= acetici acid, PRO=propionic acid, LACT=lactic acid, ETH=ethanol, n-BUT=butyric acid

The value in the round brackets is standard deviation (SE)

Table 4. Biogas and methane production, methane concentration, and VS degradation of the biomasses analyzed individually in BMP test in batch.

	Biogas yield	Methane yield	Methane content	VS degradation
	$[l_N\ kg^{-1}$ VS]		[% v/v]	[% on added VS]
RS	556.5 (6.9)	279.9 (3.3)	50.3 (0.2)	60.7 (0.9)
MS	688.3 (8.4)	347.6 (0.7)	50.5 (0.4)	66.0 (0.3)
TS	612.4 (7.9)	321.1 (3.5)	52.4 (0.1)	58.3 (1.3)

The value in the round brackets is standard deviation (SE).

Table 5. Parameters observed during CSD trials with the two selected rice straw feeds before and after feedstock extrusion.

		RS10		**RS30**	
		Biomasses		Biomasses	
		No extruded	Extruded	No extruded	Extruded
CH_4 yield	l_N day^{-1}	1.5^b	1.8^a	1.5^b	1.6^b
CH_4 specific yield	l_N kgDM^{-1} day^{-1}	146.1^c	169.0^a	140.0^c	154.9^b
CH_4 specific yield	l_N kgVS^{-1} day^{-1}	153.3^c	177.3^a	148.8^c	162.6^b
DM degradation	%	63,5^c	72,7^a	62,3^b	71,3^a
VS degradation	%	67,8^b	76,7^a	66,7^b	76,5^a
H-CEL degradation	%	82,6^b	88,7^a	82,0^b	88,3^a
CEL degradation	%	68,9^c	75,1^a	67,7^c	73,8^b
pH		7,44^a	7,33^c	7,45^a	7,39^b
Digestate DM	%	3,5	3,3	3,5	3,3
Digestate Ash	% on DM	31,3^c	31,1^c	33,2^b	34,6^a

In the same row within trial, means with the same letter are not significantly different for $P < 0.05$.