

RESEARCH ARTICLE

Environmental Engineering

Optimization of high solids batch anaerobic co-digestion of lignocellulosic biomass and cow dung under mesophilic temperature conditions

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Abstract: Anaerobic digestion is applied to recover energy from rice straw (RS) which is a lignocellulosic agricultural residue produced in huge quantities in Asia and Africa. Because of the high solids content of this feedstock, high solids anaerobic co-digestion in batch mode must be further investigated. In this study, optimal operating conditions for the anaerobic co-digestion of RS with cow dung (CD) in batch mode, with and without leachate recirculation, were assessed under mesophilic temperature conditions. Preliminary experiments carried out in 2 L batch reactors confirmed that the concentration of RS in the mixture of substrates S_0 , in g VS_{RS}/kg of mixture is an important parameter. Only batch reactors with the lowest S_0 values (29 g VS_{RS}/kg) produced biogas and rest of the reactors followed a long lag phase. The use of digestate from a previous batch as an inoculum was investigated with S_0 values of 29 and 55 g VS_{RS}/kg . Use of the digestate with S_0 of 29 g VS_{RS}/kg improved both initial degradation kinetics and the methane yield measured after 60 days. However, at S_0 of 55 g VS_{RS}/kg , the degradation kinetics were affected and after two months, 32 % of the biodegradable organic matter could not be eliminated. When leachate recirculation was performed in 6L leach-bed reactors (LBRs) with S_0 between 30 and 65 g VS_{RS}/kg , the highest methane yield was recorded at the lowest S_0 value. It can be concluded that under batch mode, an RS concentration around 30 g VS_{RS}/kg may be recommended for industrial applications.

Key words: High solids anaerobic co-digestion, leachate recirculation, lignocellulosic biomass, mesophilic.

INTRODUCTION

Rice straw (RS) is a lignocellulosic agricultural residue that is widely produced, particularly in Asia and Africa. RS is a dry material with a very high total solids (TS) and volatile solids (VS) content (He *et al.*, 2008; Contreras *et al.*, 2012) with cellulose, hemicellulose and lignin as its main components (Liotta *et al.*, 2015; Hills & Roberts, 1981). Cellulose and hemicellulose are its principal biodegradable components but the complex lignocellulosic structure and relatively high lignin content (*i.e.*, 10-15 % dry weight) increase its resistance to anaerobic biodegradation (Mussoline *et al.*, 2011; Brown *et al.*, 2012). Methane generation potentials ranging from 92 to 280 L/kg of VS have been reported in the literature for RS (Mussoline *et al.*, 2011; Achinas *et al.*, 2016) with or without pretreatment under different inocula.

The high C:N ratio of rice straw indicates a potential risk of insufficient nitrogen for bacterial growth in the case of mono-digestion of RS. Thus an external source of nitrogen is essential for the effective digestion of RS. Optimal digestion conditions, such as pH (6.5 - 8.0), temperature (35 - 40 °C), and nutrients [C:N ratio of (25-35):1] are critically important for stable anaerobic

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conversion (Mussoline *et al.*, 2011). In addition to limited moisture and nitrogen content, lignocellulosic biomass such as RS also possesses low micronutrient content. Micronutrients such as Fe and Ni are critical for enzymatic activity and the micronutrient deficiency can lead to failure of the anaerobic digestion (AD) process. Thus, the anaerobic co-digestion (AcoD) of RS is essential to overcome the problems associated with its mono-digestion (Khalid *et al.*, 2011; Hagos *et al.*, 2016). Several researchers have listed the benefits ensuing from AcoD; these include enhanced process stability, increase of methane yield, dilution of inhibitory substances, nutrient balance, adjustment of the required solids content in digester feeding, the synergetic effects of microorganisms, and an increase in the organic loading rate (Shah *et al.*, 2015; Ge *et al.*, 2016; Neshat *et al.*, 2017; Guven *et al.*, 2018). For example, the high-solids anaerobic co-digestion (HS-AcoD) of food waste (FW) and straw (maize, sorgo, and wheat) at mesophilic temperatures showed a 39.5 % increase in the methane yield of FW and a 149.7 % increase from straw compared to their mono-digestion (Yong *et al.*, 2015). In a study conducted by Brown *et al.* (2013), the HS-AcoD of food waste and yard waste also gave a 20 % increase in volumetric methane production.

Both wet anaerobic digestion (*i.e.*, TS content < 10-12%) and high-solids anaerobic digestion (HS-AD) with TS content \geq 10-12% (Abbassi-Guendouz *et al.*, 2012) can be used in the AcoD of rice straw. However, HS-AD has attracted increasing attention in recent years, especially for the digestion of lignocellulosic biomass such as wheat or rice straw. This is due to less consumption of water, no or only slow-moving parts for mixing, a lower energy requirement for heating and mixing, and easy handling of the end product (Li *et al.*, 2011; Brown *et al.*, 2012). In addition, problems arising in wet AD such as the floating and stratification of fats and fibers do not occur in HS-AD (Chanakya *et al.*, 1999).

On the other hand, poor start-up performance, incomplete mixing, the accumulation of volatile fatty acids (VFAs), a relatively low methane yield, and potential instability are considered as significant disadvantages of HS-AD of lignocellulosic biomass (Jha *et al.*, 2011; Shi *et al.*, 2013; Yang & Li, 2014; Liotta *et al.*, 2015;). Low methane yield from HS-AD can be caused by the recalcitrance of lignocellulosic biomass and by mass transfer limitations within the digester content, leading to reduction in the rate of hydrolysis, which is the rate limiting step in HS-AD of lignocellulosic feedstock. Moisture content is a critical factor in facilitating mass transfer. In HS-AD, the high solids content implies high heterogeneity that leads to slow mass transfer between microbes and substrates, resulting in possible slow

methane production and low methane yield (Kalyuzhnyi *et al.*, 2000; Bollon *et al.*, 2013; Liotta *et al.*, 2015). A high TS content of 30–35 % may even hinder solid-liquid-gas transfer, leading to accumulation of carbon dioxide (CO₂) and hydrogen (H₂) that inhibit methanogens (Bollon *et al.*, 2011).

Both continuous and batch reactors can be operated under HS-AD conditions. Continuous processes dominate among HS-AD systems treating municipal solid waste (MSW) but they are not widely applied to the processing of lignocellulosic biomass, due to mixing techniques which are not suited to HS-AcoD of lignocellulosic biomass (Li *et al.*, 2011; Degueurce *et al.*, 2016). HS-AcoD of lignocellulosic biomass such as RS presents a number of advantages when running in batch mode: relative simplicity, minimum maintenance requirement, low energy consumption, minimum capital cost. However, operating conditions of such reactors, such as temperature, pH, buffering capacity, inoculation, and control of VFAs concentration still need to be optimized in order to maximize the biogas production during a shorter retention time.

Experimental strategies such as re-use of digestate and leachate recirculation have been applied in various studies for different types of waste, including food waste and spent animal bedding. Treated spent animal bedding in batch reactors with leachate recirculation, also known as leach-bed reactors (LBRs) showed that LBRs could achieve an average of 89 % \pm 11 % of the bio-chemical methane potential (BMP) after 60 days of operation (Chugh *et al.*, 1998; Michele *et al.*, 2015; Shah *et al.*, 2015; Riggio *et al.*, 2016, 2017). According to a study conducted by using food waste as the substrate, Gottardo *et al.* (2017) found that proper leachate recirculation could control the pH value of the reactor above 5, which is important at the initial phase of the anaerobic digestion process to control acidification. Wilson *et al.* (2016) conducted anaerobic co-digestion of food waste and yard waste (leaves and grass clippings) in LBRs by combining leachate recirculation with re-use of digestate (~10 % by mass). They observed the enhancement of methane due to improvement of hydrolysis. When evaluating all these studies, authors considered basically the TS content within the reactor for the HS-AD process. But VS concentration of the highly acid producing substrate at the initial stages of the HS-AcoD process is a key parameter, as most of the reactors fail during first few days of operation.

In this study, anaerobic digestion of RS using cow dung (CD) as a co-substrate was investigated in batch mode, both with and without leachate recirculation. The aim was to define the optimal conditions to be used for

HS-AcoD of RS and CD in batch mode for application on an industrial scale. In the first part, initial conditions were studied in order to define the optimal RS and CD ratio by mass for the initiation of HS-AcoD process and continual operation, more specifically the influence of initial RS concentration (S_0). Then the digestate was reused in successive batches, and finally, experiments were conducted in batches, with leachate recirculation, in LBRs.

MATERIALS AND METHODS

Substrates

Rice straw (RS) and cow dung (CD) were used as substrates in this study. These were collected from two different farms located in the South of France. Rice straw was ground into 30-40 mm pieces using a 3 kW Blick BB230 shredder.

Experimental setups and operation

Batch reactors without leachate recirculation

All batch experiments were carried out in 2 L glass bottles. The bottles were sealed after introducing the substrate mixtures and maintained at 35 °C without agitation. All batch experiments were duplicated. Each reactor was connected to a gas collection bag (Tedlar bag, Zefon International, Inc.) in order to collect and store the biogas produced. Biogas volume in gas collection bags was measured regularly using the water displacement method. For all the reactors, the parameters monitored were biogas volume and composition. At the end of the digestion period, TS and VS concentrations of the digestate and VFA concentrations were measured.

In a preliminary experimental set-up (see Appendix 1), different mixture ratios of RS and CD were evaluated to modify the initial TS contents from 15 to 36 % and the

initial VS concentration of RS (S_0) (g VS of rice straw per kg mixture (g VS_{RS}/kg)) from 29 to 232 g VS_{RS}/kg .

The effect of inoculation on the HS-AcoD of RS and CD was investigated by re-using the digestate from one batch to the next one. Three successive batch reactors named Batch 1, Batch 2 and Batch 3 were operated using different substrate quantities, and the compositions are as shown in Table 1. Batch 1 was operated without an external source of inoculum, and operating conditions of this reactor is similar to the reactor that produced methane in the preliminary experiment (see Appendix - 1). At the end of the digestion period, digestate recovered from the Batch1 reactors was used as an inoculum source for Batch 2 reactors; similarly, digestate recovered from Batch 2 reactors was used as the inoculum for Batch 3 reactors. In Batch 1 and Batch 2 reactors, initial TS content of the mixture was 15 % and 16 %, respectively. Initial VS concentration of RS (S_0) in the mixture was same (29 g VS_{RS}/kg) in these two reactors. In Batch 3 reactor, both TS content and S_0 were increased to 20 % and 55 g VS_{RS}/kg , respectively.

Leach-bed batch reactors

Four leach-bed batch reactors (LBRs) made of glass (14.5 cm internal diameter, 45 cm high) with a working volume of 6 L were used (Riggio *et al.*, 2017). In order to retain the leachate at the bottom of the each reactor, the solid and liquid fractions were kept apart by a mesh (1 mm holes) placed at 10 cm up from the bottom of the reactor. Leachate was re-circulated using a peristaltic pump. In order to equalize the pressure between the two compartments, the headspace and the volume below the mesh were connected by using a tube. Each reactor was connected to a water bath and temperature was kept at 35 °C by recirculating water through the jacket. Each reactor was also equipped with a Ritter gas flow meter (MGC-1 V3.1 Milligascounter) to automatically measure the biogas volumes, and a gas collection port

Table 1: Experimental conditions for successive batches

	Batch 1	Batch 2	Batch 3
RS (g)	25	25	50
CD (g)	650	350	480
Solid digestate (g)	-	300	190
Initial TS content of mixture (%)	15	16	20
S_0 (g VS_{RS}/kg)	29	29	55

was available in each reactor to collect biogas for subsequent composition analysis. A leachate collection port was connected to the leachate stock of each reactor in order to collect leachate for pH measurement and VFA analysis (Riggio *et al.*, 2017).

Inoculation and leachate preparation

Solid and liquid fractions of digestate were collected from a batch digester installed at a farm which uses lignocellulosic substrate and cow manure as the feedstock for the first LBR experiment. This digestate was used as the inoculum and for liquid recirculation. The TS and VS content of the solid fraction of the digestate were 17.0 % and 11.8 %, respectively. The TS and VS content of the liquid fraction of the digestate were, 4.5 % and 2.6 % respectively, and alkalinity, total VFA concentration, and pH were 17.9 g CaCO₃/L, 743 mg COD-VFA/L, and 8.5, respectively. The leachate used for recirculation within the LBRs was prepared by mixing the liquid fraction of the digestate with water at a ratio of 1:1 by Volume.

Operating conditions

Two sets of experiments were carried out using LBRs. In the first set, four 6 L leach-bed reactors were used. The first three reactors were run with different quantities of rice

straw in order to increase the initial VS concentration of rice straw (S_0). Rice straw, cow dung and solid digestate were mixed together to obtain initial S_0 concentrations in each reactor of 30, 36, and 47 g VS_{RS}/kg, and an initial TS content of around 12.5 %. Substrate-to-inoculum (S/I) ratios in a VS basis (rice straw VS mass / solid digestate VS mass) were 0.7, 1 and 2, respectively (Table 2). The fourth reactor was operated only with digestate and served as a control reactor.

After filling each reactor with the prepared mixture of substrate, a 7 kg weight was applied to the top of each substrate mixture for 10 min in order to reduce its bulk volume. Then the reactors were closed and, the leachate was re-circulated through the top of the reactors for 5 min using a peristaltic pump to saturate the bulk volume with water. No further leachate recirculation was carried out in the control reactor. For other three reactors, leachate recirculation was set automatically, once in every 12 h at a rate of 600 mL/min for 2 mins. After 55–60 ds, all the reactors were opened and the solid digestate and leachate samples were collected.

The solid digestate obtained from each reactor of the first set-up, except from the control reactor, were mixed together with RS and CD to prepare mixtures of substrate and inoculum for the second set of LBR experiments. For

Table 2 : Quantities of substrate, digestate and water added for first and second LBR experiments

Reactor	1 st LBR experiment (P-1)				2 nd LBR experiment (P-2)			
	1-(1)	1-(2)	1-(3)	1-(4) (Control)	2-(1)	2-(2)	2-(3)	2-(4) (Control)
RS (g)	132	156	205	0	205	250	300	0
CD (g)	450	600	778	0	771	900	1000	0
Solid digestate (g)	1262	1044	686	2500	779	950	1139	2865
Liquid digestate (g)	820	830	900	500	850	760	615	500
Water (g)	820	830	900	500	850	760	615	500
S_0 (g VS _{RS} /kg)	30	36	47	0	47	55	65	0
Global TS content (%)	12.3	12.5	12.7	12.8	13	14.7	16.9	10.8
S/I (VS mass rice straw/VS mass solid digestate)	0.7	1	2	-	2	2	2	-

the first three reactors, solid digestate recovered from first LBRs experiment, RS and CD were mixed together to obtain initial rice straw VS concentration (S_0) values of 47, 55, and 65 g VS_{RS}/kg , respectively. Initial TS content and S/I ratio (VS basis) were kept at 13–17 % and 2, respectively, for the three reactors (Table 2). The remaining digestate was used for the fourth reactor which was operated as the control reactor. Operations, monitoring and analysis were carried out in a manner similar to previous experimental setup.

Analytical methods and experimental protocols

The total solids (TS) and volatile solids (VS) content, as well as the alkalinity of the substrates and digestate were measured in accordance with the APHA standard methods (APHA, 2005). Triplicates of each substrate sample were taken in order to obtain average values of TS and VS percentages.

Biochemical methane potentials (BMPs) of rice straw and cow dung were evaluated using a protocol described by Riggo *et al.* (2017).

For all experiments, biogas composition was analyzed using gas chromatography (GC Perkin 580) in which argon gas was used as the carrier gas. For the LBRs, pH of the leachates collected at leachate collection ports were measured using a pH probe. VFAs were analyzed using gas chromatography (Perkin-Elmer Clarus 580 GC) with N_2 as the carrier gas.

RESULTS AND DISCUSSION

At the beginning, both rice straw (RS) and cow dung (CD) were characterized. TS and VS contents of RS were 92.8 % and 79.4 % respectively and 12.3 % and 10.6 % respectively for CD. BMP of RS and CD were 254 ± 11 and 173 ± 1 NmL/g VS. The BMP value of RS was almost 50 % higher compared to that of CD. This lower BMP value of CD indicates that the biodegradable volatile-solids fraction in the CD was lower than that in the RS. It can be determined that a certain fraction had already been digested via the digestive system of the cows.

Co-digestion of RS and CD in batch reactors

The main objective of conducting batch anaerobic digestion in preliminary experiments (Appendix 1) was to evaluate the inoculation efficiency of cow dung and understand the effect of the initial VS concentration (S_0) of rice straw for HS-AcoD process of lignocellulosic biomass.

Only the reactor operated with the lowest S_0 of 29 g VS_{RS}/kg produced biogas. High substrate concentrations and particularly high RS concentrations, led to digester failure by acidification. In addition, it was observed that the effectiveness of cow dung as a source of inoculum was rather poor as this reactor had a 15-day lag phase and quite low degradation rates. Three successive batches were treated under the operating conditions presented in Table 1. In order to evaluate the effect of inoculation, digestate from the 15 %TS reactor (Batch 1) was re-used as an inoculum for a Batch 2 reactor whose operating conditions remained very similar to Batch 1 (*i.e.*, TS content of 16 % and the same S_0 concentration of 29 g VS_{RS}/kg). For the Batch 3 reactor, digestate of the Batch 2 reactor was re-used as inoculum, but a higher TS content of 20 % and a higher S_0 concentration of 55 g VS_{RS}/kg were maintained in order to investigate the impact of higher S_0 concentrations.

Figure 1 (a) and 1 (b) present, respectively, the evolution of methane production and the methane yield over time for the three successive batches. Methane production in each reactor was corrected by deducting the contribution to methane production by the digestate alone. To normalize methane yield in each reactor it was calculated based on the quantity of volatile solids added from the substrates to each reactor. Therefore, VS added in the calculation consists only of VS from RS and CD.

In Batch 2 reactor, almost 50 % of the cow dung in the initial mixture was replaced by digestate from the first batch. The re-use of digestate as an inoculum enhanced the anaerobic digestion performance. The lag-phase period was drastically reduced, from 15 ds to almost 0 ds (Figure 1), and a 103 % increase in the methane yield was achieved.

From the individual BMP values of RS and CD, the expected cumulative methane volume and methane yield of the second batch were 11.1 L and 202.7 mL CH_4/g VS, respectively, compared with the experimental values of 12.1 L and 221.8 mL CH_4/g VS, respectively. The difference between the expected and actual values was less than 10 % for both parameters. This suggests that all the added biodegradable VS had been eliminated under the experimental conditions of the second batch.

In the Batch 3 reactor, both TS % and S_0 were increased compared to Batch 1 and Batch 2 reactors. However, compared to the Batch 2 reactor, only an 18 % increase in total methane production was observed after almost two months. As a consequence, the methane yield was much lower in the Batch 3 reactor compared to the Batch 2 reactor. The expected methane yield calculated

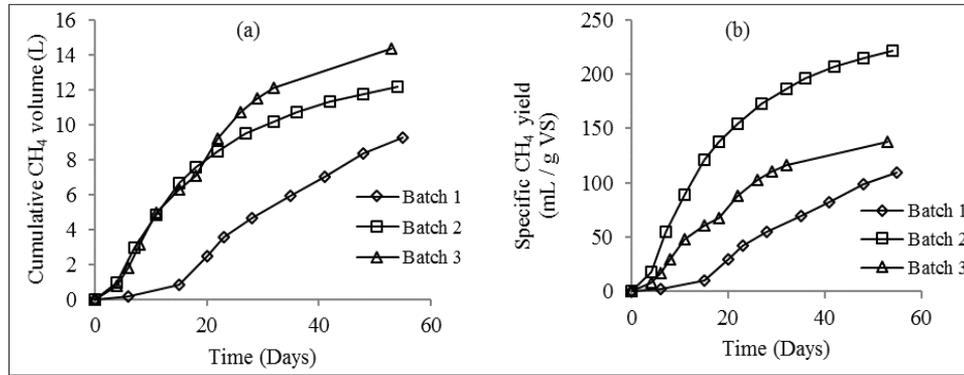


Figure 1: Evolution of cumulative CH₄ (a) and specific methane yield (b) over time of 2 L batch reactors, for the three successive batches

for the Batch 3 reactor was 203.6 mL CH₄/g VS whereas the experimental value was 137.7 mL CH₄/g VS which indicates that only 68 % of the biodegradable organic matter had actually been degraded. This lower methane production in the Batch 3 reactor can be explained by two factors: the higher TS concentration of the initial mixture and the higher initial RS concentration (S_0). In the first part it has been shown that the initial VS concentration of rice straw within the substrate mixture is a critical parameter and in the Batch 3 reactor this concentration was too high, *i.e.*, 55 g VS_{RS}/kg. Furthermore, data from the literature suggests that an increase in the TS concentration of the digestate could lead also to a negative impact on the performance of anaerobic digestion.

Abbassi-Guendouz *et al.* (2012) investigated the effects of TS on AD by using cardboard as a substrate and found that the VS-based methane production rate decreased when the reactor TS increased from 10 % to 35 %. Similarly, Motte *et al.* (2013), using wheat straw as a substrate, also showed declining VS-based methane production rate as TS increased from 15 % to 25 %. Xu *et al.* (2014) also investigated the effect of TS content on the anaerobic digestion of rice straw and found that when TS % varied between 15 % to 20 %, methane production rate increased. When exceeding this threshold limit, it decreases. TS content is a key parameter that affects the mass transfer between gas-liquid-solid phases in HS-AcoD. This parameter affects the rate of substrate degradation and the accumulation of inhibitors such as VFAs (Abbassi-Guendouz *et al.*, 2012; Xu *et al.*, 2014; Liotta *et al.*, 2015). TS content has been assumed to affect the hydrolysis rate constant, the maximum microbial growth rate and diffusion constant as well (Xu *et al.*, 2014).

In conclusion, the results obtained from the batch experiments including preliminary experiments

suggested that not only the TS content, but the initial rice straw VS concentration (S_0) is also a critical parameter, which leads to acidification and failure of the reactors. Thus it is suggested that initial S_0 values should not exceed 30 g VS_{RS}/kg.

Co-digestion of RS and CD in leach bed reactors

Leachate re-circulation is a strategy that has been using in high-solids batch anaerobic digestion to control production of VFAs at start-up in reactors known as LBRs. From the physico-chemical point of view, leachate recirculation is used to increase the moisture content of the bulk of the substrate, thereby improving mass transfer and access to organic matter (Chanakya *et al.*, 1999; Jha *et al.*, 2011). Leachate recirculation leads to leaching out the VFAs produced, to diluting inhibitor compounds, and to increasing the buffer capacity of the medium. From a biological point of view, increasing moisture content improves the growth of microorganisms (Batstone *et al.*, 2002). This will result in higher biodegradation of lignocellulosic substrates. Furthermore, the re-use of digestate facilitates improvement of the start-up of the reactor because it was possible to recycle already acclimatized biomass to the substrate.

In this study, two sets of experiments were carried out to investigate the effect of leachate re-circulation and the initial RS concentration, on the high-solids anaerobic co-digestion of RS and CD in LBRs. In the first set of experiments, three conditions were tested with initial rice straw VS concentrations (S_0) at 30, 36 and 47 g VS_{RS}/kg (Table 2). In the second set of experiments, initial rice straw VS concentrations of 47, 55 and 65 g VS_{RS}/kg were tested (Table 2). The maximum reaction time for all conditions was fixed at 60 days. In these experiments, the quantities of RS and CD were increased but the proportion of RS and CD in terms of VS remained very close, with

rice straw VS representing on average 67.4 ± 1.3 % for the six mixtures used. As a result, the accumulated volume of methane produced from RS represented 75.2 ± 1.1 % of the expected total volume of methane produced and the average expected methane yield of the mixtures was 228 ± 1 mL CH₄/g VS. The only difference between the two sets of experiments was the digestate used for inoculation. In the first experiments, digestate was sampled from an industrial-scale LBR whereas, for the second experiment, digestate collected at the end of the first experiment was used.

Methane production

Figure 2 (a) and 2 (b) present the total volume of methane produced by the LBRs and the methane yield, respectively, as a function of the initial VS concentration of rice straw (S_0). The initial RS concentration was selected to plot these curves due to three reasons: (i) 75 % of methane volume is produced from RS; (ii) organic matter of RS has the highest degradation kinetics; and (iii) it was shown previously in batch mode that the initial VS concentration of rice straw (S_0) is a critical parameter in reactor acidification and failure.

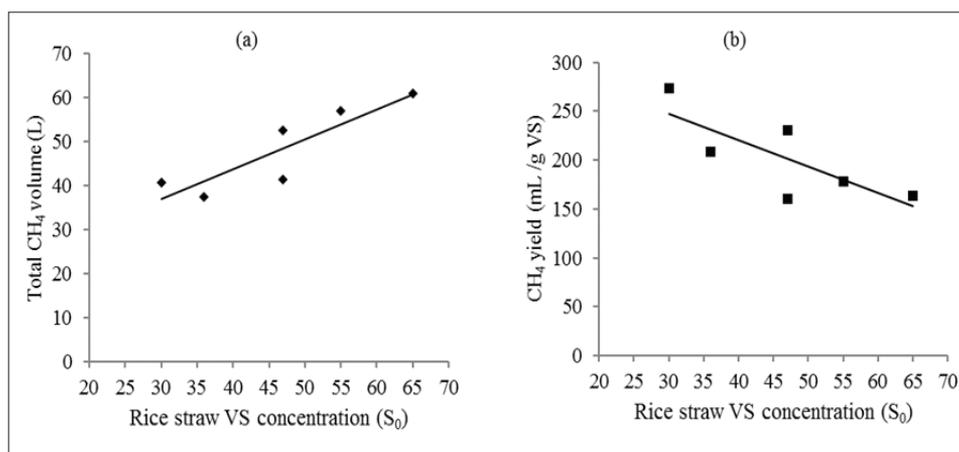


Figure 2: Evolution of the total volume of CH₄ produced (a) and of the specific methane yield (b) over the quantity of rice straw VS added for the experiments in LBRs

Figure 2 (a) shows that total methane production increased with increasing S_0 values. However, this increase was not proportional to the added quantity of VS. For example, if the two extreme values are compared, only an additional 50 % of CH₄ was produced when the initial RS quantity was doubled. As a consequence, the methane yield decreased when S_0 was increased. For the lowest quantities of substrate added, the methane yield observed in LBRs (240 ± 32 mL CH₄/g VS) was close to the BMP of the mixtures calculated from the BMP of RS and CD (228 ± 1 mL CH₄/g VS) whereas, for the highest substrate load, the methane yield was only 70 % of the expected value, indicating that during the two months period, all the organic matter added was not degraded.

VFAs concentration, pH and alkalinity

Total concentration of VFAs and pH were measured regularly in the liquid fraction, *i.e.*, leachate of each reactor. Figure 3 presents the variation of total VFA and

pH throughout the digestion period. VFAs production was high at the beginning of the batch experiments indicating that methanogenesis was slower than acidogenesis.

The maximum total concentration of VFAs in each reactor was reached after 5-6 days of operation, ranging between 6000 and 12000 mg COD-VFA/L (Figure 3 (a) and 3 (c)). At the same time, pH decreased as shown in Figure 3 (b) and 3 (d) but always remained above 6.6 due to the high alkalinity of the mixture of digestate and substrates (above 8.3 g CaCO₃/L).

VFA concentrations of the leachate dropped rapidly to almost 0 mg/L before day 20. Several authors have reported similar behaviors. Mussoline *et al.* (2012) have observed an initial peak of VFA in terms of acetic acid (3375 mg HAc/L) which occurred at the beginning of the experiment on dry AD of rice straw and piggery wastewater. In this study, at day 57, VFA concentration dropped below 200 mg HAc/L. Similar observations

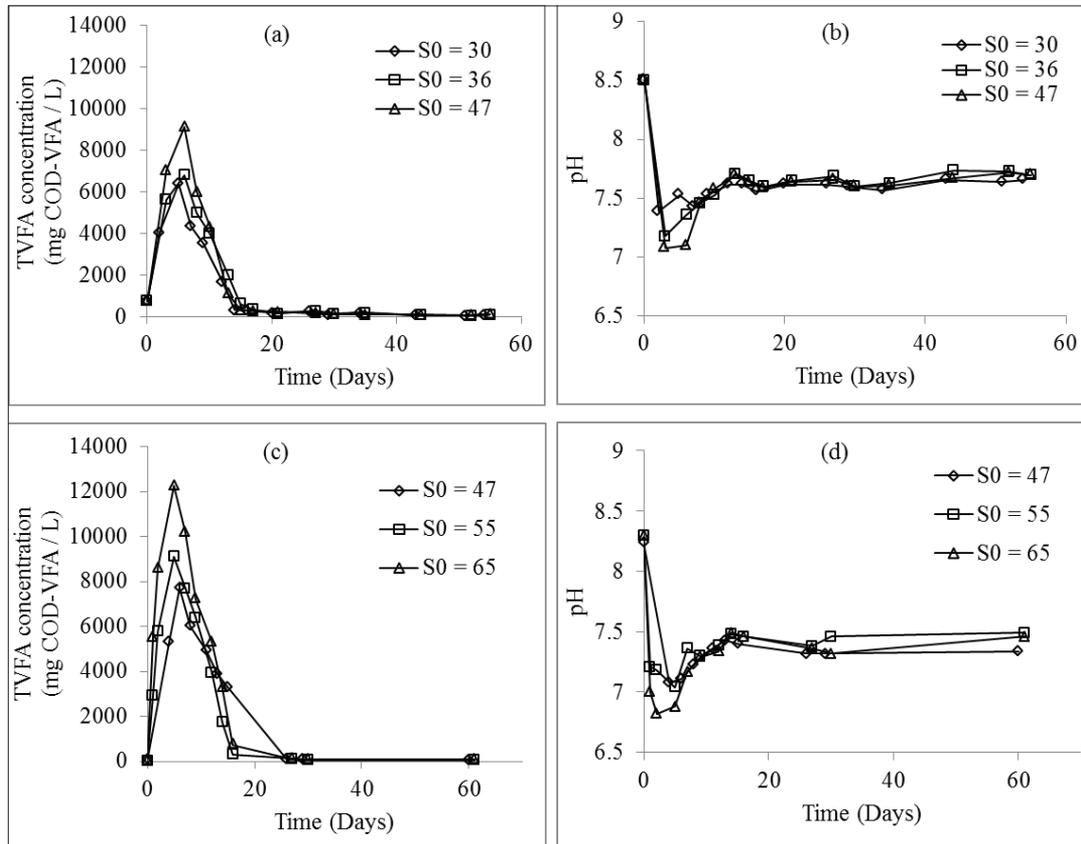


Figure 3: Total VFA and pH variation in the first (a and b) and second (c and d) LBR experiments, over time

could be found in the study on hybrid solid anaerobic digestion of the organic fraction of municipal solid waste conducted by Massaccesi *et al.* (2013). From these results, the stable operation of the reactors and the optimization of the leachate recirculation time period can be identified as highly important factors when these types of reactors are operated on a large scale.

Effect of leachate re-circulation

In order to evaluate the influence of leachate recirculation, results obtained without recirculation in pure batches, *i.e.*, 2 L bottles of successive batches and with recirculation in 6 L LBRs were compared at two initial RS concentrations of 30 and 55 g VS_{RS}/kg. In terms of methane yield, the performances observed in LBRs were slightly better than those in 2 L batch reactors without recirculation. In terms of specific methane yields, it was 273 mL CH₄/g VS in the LBR compared to 222 mL CH₄/g VS in the 2 L batch reactor (a 23 % increase) at 30 g VS_{RS}/kg; and 179 mL CH₄/g VS in the LBR compared to 138 mL CH₄/g VS in the 2 L batch reactor (a 30 % increase) at 55 g VS_{RS}/kg. Specific methane production rates are shown in Figure 4. At S₀ of 30 g VS_{RS}/kg, specific methane production rates

were slightly higher in the LBR compared to the batch reactor at the beginning of the operation. However, after 1 month of operation, the slowly-biodegradable organic matter had similar rates of degradation. At S₀ of 55 g VS_{RS}/kg, rates were quite close except during the period 10 - 20 days, during which lower rates were reported for the batch without recirculation. This is due to some partial inhibition in that period resulting from VFA production and a drop in pH. After day 20, rates once again showed similar trends.

As already shown, both specific methane production rates and specific methane yields dropped with the increase of initial rice straw VS concentration. In conclusion, leachate recirculation slightly improved both performance and stability of the reactors, but operation in a pure batch still remained possible under the operating conditions applied.

Optimal operating conditions for LBRs

From the results obtained in the two sets of experiments with LBRs, it was possible to assess the optimal operating conditions of LBRs treating a mixture of RS

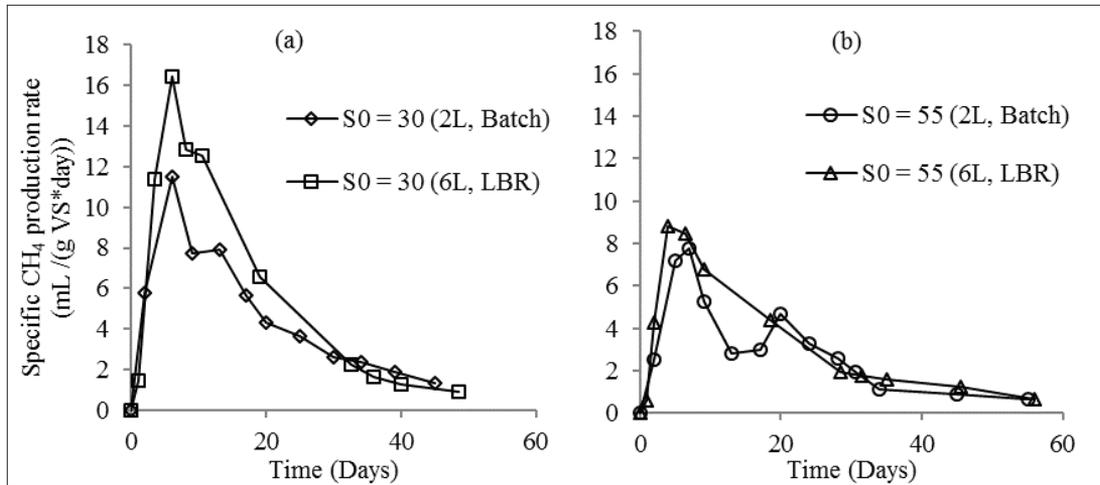


Figure 4: Specific methane production rates: comparison of 2 L batch reactors and 6 L LBRs that have S₀ (a) value of 30 and (b) 55 g VS_{RS}/kg

and CD. Figure 5 clearly demonstrates that the maximum total VFA concentration increased with the increase of initial rice straw concentration (S₀); however reactors were operated safely even at higher S₀. This is due to the buffering capacity provided by the leachate and the washout of VFAs by leachate recirculation. However, use of higher initial RS concentrations might have led to potential inhibition at the beginning of the batch. It could be noted that the maximum total concentration of VFAs measured at the beginning of the batch with a S₀ of 65 g VS_{RS}/kg was 12,200 mg COD-VFA/L and the minimum pH was 6.82, which is close to the critical value for methanogenesis inhibition of 6.6 (Batstone *et al.*, 2002; Mussoline *et al.*, 2011)

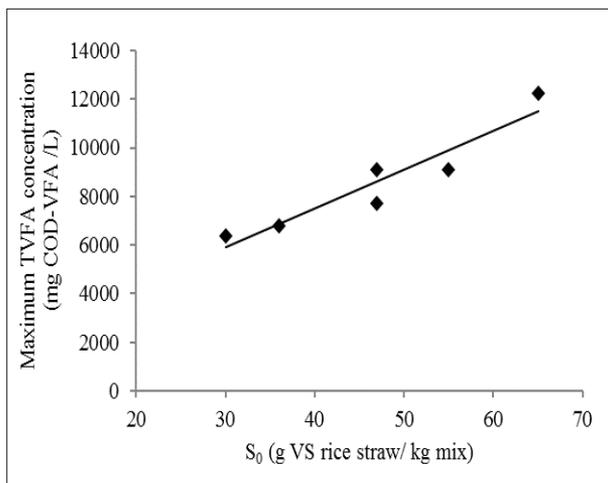


Figure 5: Maximum total volatile fatty acids concentration in LBRs with initial VS concentration of rice straw (S₀)

Furthermore, specific methane yield decreased as substrate loads increased (Fig. 2 (b)) and VS removal for the highest substrate load was only 70 % of the calculated value after two months of operation. As a result, in order to obtain optimal methane production in LBRs, along with the maximal VS reduction and operational safety, the recommended initial RS concentration S₀ in the mixture should be at a maximum of 30 g VS_{RS}/kg.

The results obtained in this study also suggest that leachate recirculation can be stopped after 15 days of operation as total VFA production at this time had drastically decreased and the accumulated VFA concentration was below 740 mg COD-VFA/L, even for the highest substrate load. André *et al.* (2015) also found that during the dry AD of cattle manure, leachate recirculation had no effect on methane production rates after 19 days of degradation.

CONCLUSIONS

In this study, the anaerobic co-digestion of mixtures of rice straw and cow dung was studied both in pure batch reactors and batch reactors with leachate recirculation. Preliminary experiments carried out with RS and CD alone at different initial concentrations showed a high risk of acidification at the higher substrate concentrations and low efficiency and effectiveness of cow dung as a source of inoculum. Therefore, two strategies were investigated: first, the re-use of digestate for inoculation in the pure batches and, second, leachate recirculation using leach-bed reactors (LBRs). When the digestate from a previous batch is used as an inoculum, the lag phase for

methane production in batch was reduced from 15 days to almost none and the specific methane production rates increased significantly. Furthermore, after 55 days of digestion, a 103 % increase of the cumulative volume of methane produced was achieved in 16 % TS batch reactors ($S_0 = 29$) operated with the digestate re-use strategy, compared to the reactor fed only with a mixture of rice straw and cow dung. In terms of methane yield, the performances observed in LBRs were slightly better than those in the batch reactors with a 23 % increase for the S_0 of 30 g VS_{RS}/kg and 30 % increase for the S_0 of 55 g VS_{RS}/kg . However, specific methane yields and specific methane production rates dropped with the increase of lignocellulosic substrate concentration within the reactors. For optimal methane production in batch and in LBRs, as well as for operational safety, initial RS concentration in the mixture S_0 should be at a maximum of 30 g VS rice straw/ kg mixture. Therefore, further investigations should be conducted to ascertain the threshold values (*i.e.*, TS content and S_0) for optimizing the HS-AcoD of lignocellulosic biomass.

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Appendix-1

Preliminary experimental setup

In this experimental setup, four different initial TS contents were used: 36, 29, 22 and 15%. To obtain the different TS percentages, two substrate mixing strategies were applied. In the first strategy (Mixing strategy-1), only rice straw (RS) and cow dung (CD) were mixed together to obtain the relevant TS% in the mixture. As a consequence, the quantity of CD was increased to reduce the initial TS content of mixture. The quantities of RS and CD added are presented in Table 1. In the second strategy (Mixing strategy-2), the quantities of RS and CD were identical in all the batch reactors (i.e. corresponding to the TS content of 36% of Mixing strategy-1) and water was added to fix the percentage of TS. The quantities of RS, CD and water added are presented in Table 1. The initial VS concentration of RS (S_0) in g VS of rice straw per kg mixture (g VS_{RS}/kg) was also calculated; it was between 29 and 232 g VS_{RS}/kg .

Table 1: Experimental conditions for the preliminary experimental setup

	Mixing strategy-1 (E-1)				Mixing strategy-2 (E-2)			
	36	29	22	15	36	29	22	15
Initial TS content of mixture (%)	36	29	22	15	36	29	22	15
RS (g)	50	50	50	25	50	50	50	50
CD (g)	120	188	355	650	120	120	120	120
Water (g)	-	-	-	-	-	40	107	236
S_0 (g VS_{RS}/kg)	232	166	98	29	232	188	143	97

Figure 1 (a) shows the evolution of cumulative methane volumes produced over time for all the eight conditions tested. The only reactor which actively produced biogas was the 15% TS reactor which contained a mixture of 25 g RS and 650 g CD. Furthermore, the same reactor also showed a 15-day lag phase. None of the other reactors produced any significant volumes of biogas over the 80 days. In addition, Fig. 1 (b) shows that the final VFAs concentration in all these non-producing reactors was very high and rose with the increase of the initial TS percentage in both feeding strategies. This shows that strong acidification occurred in the reactors, except for the batches carried out at 15% TS and at the lower initial S_0 of 29 g VS_{RS}/kg .

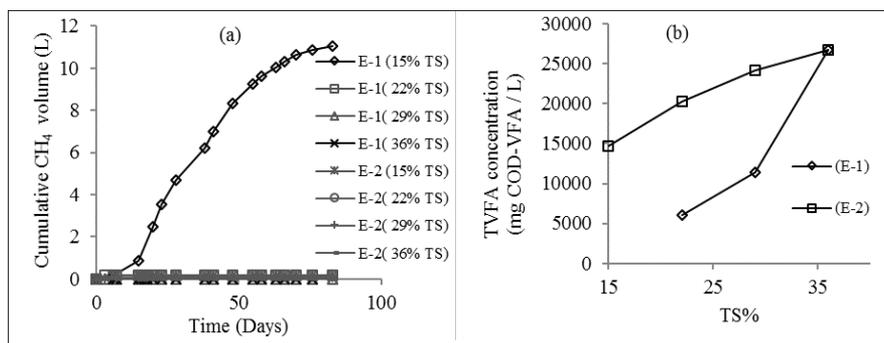


Figure 1: Cumulative methane volumes produced (a) over time and final total volatile fatty acids concentrations (b) in the preliminary experiment