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Short Communication

Innovative two-stage anaerobic process for effective codigestion of cheese whey and cattle manure

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HIGHLIGHTS

- ▶ A two-stage anaerobic process for enhancing organic waste codigestion was studied.
- ▶ The innovative two-stage process designed for reducing footprint.
- ▶ Cheese whey and cattle manure were codigested.
- ▶ The highest methane yield was obtained co-treating the two wastes at equal ratio.
- ▶ The proposed system improved the methanisation yield.

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ABSTRACT

The valorisation of agroindustrial waste through anaerobic digestion represents a significant opportunity for refuse treatment and renewable energy production. This study aimed to improve the codigestion of cheese whey (CW) and cattle manure (CM) by an innovative two-stage process, based on concentric acidogenic and methanogenic phases, designed for enhancing performance and reducing footprint. The optimum CW to CM ratio was evaluated under batch conditions. Thereafter, codigestion was implemented under continuous-flow conditions comparing one- and two-stage processes. The results demonstrated that the addition of CM in codigestion with CW greatly improved the anaerobic process. The highest methane yield was obtained co-treating the two substrates at equal ratio by using the innovative two-stage process.

The proposed system reached the maximum value of 258 $mL_{CH_4}\,g_{VS}^{-1}$, which was more than twice the value obtained by the one-stage process and 10% higher than the value obtained by the two-stage one.

1. Introduction

The proper management and valorisation of agroindustrial waste (i.e. organic waste) through anaerobic processes represents a significant opportunity to combine waste treatment and renewable energy production (Esposito et al., 2012).

CW is the main by-product of the dairy industry. It is characterised by a very high organic load and low buffer capacity; consequently, the direct anaerobic treatment of raw whey can lead to rapid acidification which results in low biogas productivity (Ghaly, 1996; Malaspina et al., 1996; Saddoud et al., 2007). Contrary to CW, CM is characterised by low C/N ratio (Esposito et al., 2012). Owing to the high nitrogen content of CM, ammonia tends to accumulate in digesters resulting in anaerobic digestion (AD) microbial processes inhibition (Nielsen and Angelidaki, 2008). Several studies have demonstrated that the codigestion of CW with CM can maintain favourable pH and improve biogas production (Gelegenis et al., 2007; Ghaly, 1996) but the optimal co-substrates ratio and the theoretical gas yield greatly vary according to each specific case (Esposito et al., 2012; Saddoud et al., 2007).

AD is not widespread in the dairy industry because CW normally displays high acidification potential and requires long hydraulic retention times (HRTs), and because of the small scale and fragmentation of dairy factories. Over the last decade, various bioreactor configurations have been evaluated and proposed for biogas improvement from organic waste (Nasir et al., 2012; Nizami and Murphy, 2010). Igoni et al. (2008) stated that simple and

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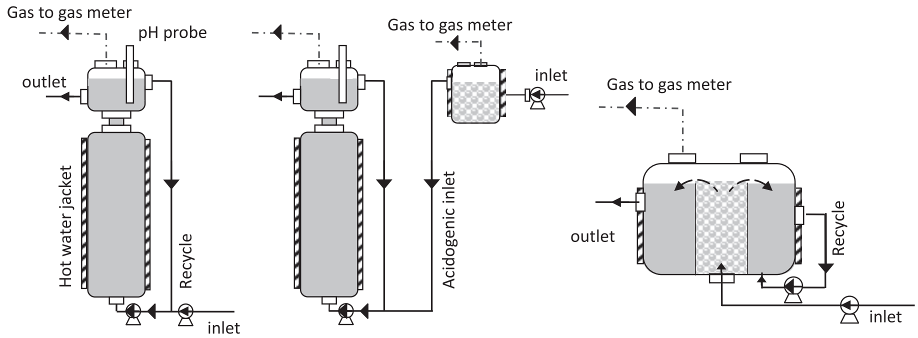

Fig. 1. Schematic diagrams of the three reactors: (a) one-stage reactor (R1); (b) two-stage reactor (R2); (c) two-stage concentric reactor (R3). (Not drawn to scale.)

reduced design systems suffer less frequently from technical failures, and this results in economic benefits because of the reduced costs in process design, construction and management. On the contrary, the codigestion and two-stage reactors can provide higher treatment efficiency and process stability in relation to the single-substrate digestion and the use of one-stage processes (Nasir et al., 2012; Saddoud et al., 2007).

This study aimed to develop an innovative two-stage process devoted to the co-digestion of CW and CM, which could combine the advantages of the simplicity of conventional processes with the high efficiency of multistep reactors. To this end, a simple continuous two-stage process was designed and developed, so that the first acidogenic stage was directly inserted into the methanogenic vessel with a concentric design (Fig. 1) in order to reduce the footprint. The latter was compared with conventional one- and two-stage processes (Fig. 1). To the best of the authors' knowledge, this is the first study that aims to improve the codigestion of CW and CM by evaluating different design configurations.

2. Methods

2.1. Substrates and inocula

The agro-zootechnical wastes used as substrate (S) sources for the anaerobic codigestion experiments were cheese whey (S_{CW}) and cattle manure (S_{CM}), obtained from a dairy factory and a cattle farm, respectively, both located in the Emilia-Romagna Region (Northern Italy). Since CM was provided in a semi-solid state, before being used, it was diluted with tap water (1 manure:2 water, v/v) and sieved (53-μm opening).

Three different inocula were tested as seed for the anaerobic digestion experiments. They consisted of methanogenic consortia from (a) an olive mill (I_{OM}) wastewater collected from an olive mill located in the Liguria Region (Northern Italy), (b) manure from the same cattle farm (I_{CM}) cited above and (c) sludge from a bench-scale reactor (I_R) treating organic fraction municipal solid waste as described in Bertin et al. (2012). The main characteristics of the substrates and inocula are reported in Table 1.

2.2. Batch tests

Batch tests were carried out as preliminary investigation to identify the optimal operating conditions for the anaerobic codigestion to be applied in the continuous reactors.

The methanogenic activity of the single wastes and in codigestion was measured by the biochemical methane potential (BMP; Owen et al., 1979) with minor modifications as described in Bertin et al. (2012). The tests were conducted in triplicate in 100 mL Pyrex-glass bottles started-up by adding 5 mL of inoculum and 50 mL of substrate consisting of the codigestion waste mixtures. The bottles were incubated at 35 ± 0.5 °C. The monitoring was carried out until complete methane production depletion (up to 100 d).

Three series of batch test were performed. The first series was conducted to evaluate the activity of the three inocula (I_{OM}, I_{CM} and I_R) on the two substrates (S_{CW} and S_{CM}) tested in codigestion (in equal volume ratios). The second BMP test series was conducted to identify the optimal mix ratio of the two substrates using I_R as inoculum; therefore, the two substrates were digested at different S_{CW}:S_{CM} ratios ranging from 0% to 100% v/v at progressive variations of 10%.

The third set of batch tests aimed to evaluate the optimal acidogenic stage conditions to start up the two-stage process. The experiments were performed for the short-term biochemical hydrogen potential (BHP) test as described by Giordano et al. (2011) with minor modifications. Contrary to the BMP tests, the pH was initially adjusted to 6.0 ± 0.5 by diluted HCl to improve the acidogenesis. The tests lasted for two weeks and ceased when methane was observed in the biogas. Moreover, contrary to Giordano et al. (2011), the inoculum (I_R) was not subjected to any "hydrogen-production" pretreatment. The reactors were fed with 50:50-S_{CW}:S_{CM} volumetric ratio. The acidogenesis stage was

Table 1
Main characteristics of substrates and inocula (mean ± standard deviation).

Parameter	Inoculum			Substrate		
	I_{OM}	I_{CM}	I_R	S_{CW}	S_{CM}[a]	Feed[b]
Density (g mL^{-1})	1.0 ± 0.01	1.06 ± 0.02	1.02 ± 0.01	0.99 ± 0.13	0.99 ± 0.01	–
pH	–	–	–	5.0	7.9	7.1
$_{S}$COD (g L^{-1})	22.4 ± 1.5	12.0 ± 1.5	22.6 ± 3.0	58.5 ± 1.7	9.4 ± 0.1	35.2 ± 6.7
Tot carbohydrates (g L^{-1})	–	–	–	42.2 ± 2.8	1.3 ± 0.31	11.8 ± 4.2
Proteins (g L^{-1})	–	–	–	1.3 ± 0.4	0.4 ± 0.1	0.8 ± 0.2
TS (g L^{-1})	11.5 ± 0.2	23.2 ± 4.1	31.8 ± 3.8	57.8 ± 7.9	25.6 ± 0.1	36.3 ± 2.5
VS (g L^{-1})	4.9 ± 0.1	13.2 ± 2.8	14.6 ± 1.2	52.8 ± 7.6	17.6 ± 0.1	30.4 ± 3.6

[a] Sample diluted with water (1:2).
[b] S_{CW}:S_{CM} – 50:50 v/v.

evaluated at room temperature (approximately 20 °C, i.e. without temperature control) and mesophilic (35 °C) conditions.

The batch tests were monitored daily during the first 4–6 experimental days and weekly afterwards.

2.3. Continuous codigestion experiments

The experiments were carried out using three bench-scale reactors, where one- and two-stage processes were set-up. The one-stage process was studied in a completely mixed reactor (R1, Fig. 1a). The two-stage process was investigated using two different designs: in the first design (R2), a second smaller completely mixed reactor was added before the methanogenic vessel (Fig. 1b). The second two-stage reactor (R3) consisted of a single container, which included both the acidogenic and the methanogenic stages. The former was concentrically integrated into the latter, so that the acidified effluent was fed by gravity into the methanogenic phase (Fig. 1c).

The methanogenic reactors had working volumes of 500 mL for R1 and R2, and 790 mL for R3; the acidogenic phase had a working volume of 120 and 190 mL in the R2 and R3, respectively. The three reactors were fed using peristaltic pumps set to ensure hydraulic retention times (HRTs) of 20 d for the methanogenic phase and of 5 d for the acidogenic phases, resulting in organic loading rates (OLRs) of 1.8 and 1.7 $kg_{COD}\,m^{-3}\,d^{-1}$ for the one- and two-stage reactor, respectively. On the basis of the results obtained by batch tests (see Section 3.2), the reactors were inoculated by using anaerobic sludge I_R and fed by the substrate ratio of 50:50 (v/v S_{CM}:S_{CW}). The bench-scale plants were maintained at 35 ± 1 °C.

Before starting the experiments, the reactors were operated for approximately one month in order to acclimate the biomass to the substrate. The reactors were operated for more than two months and their performance was evaluated under (almost) steady-state conditions, assumed as performance variations (in terms of COD removal rate) of less than 15%.

2.4. Analytical methods

Total solids (TS), volatile solids (VS) and soluble chemical oxygen demand (sCOD) were measured according to standard methods (APHA, 2005). Carbohydrates were estimated according to Dubois et al., (1956) and proteins were estimated using the Bio-Rad Protein Assay.

The biogas produced by the three reactors was measured by home-made gas-meters. Biogas composition, volatile fatty acids (VFAs) and pH were measured as described in Bertin et al., (2012).

3. Results and discussion

3.1. Substrates characterisation

S_{CW} and S_{CM} were different mainly due to their content of organic matter and pH (Table 1). In fact, S_{CW} had higher concentrations of carbohydrates and proteins than S_{CM} (Table 1). On the contrary, S_{CM} had a pH that was significantly higher than that of S_{CW} (Table 1). Therefore, as also proposed by other authors (Dareioti et al., 2009; Gelegenis et al., 2007; Kavacik and Topaloglu, 2010), the addition of CM to CW in codigestion can result in more robust and effective AD (Esposito et al., 2012).

3.2. Batch tests

The first set of batch tests was performed to evaluate three different inocula. The BMP experiments resulted in methane yields of 26 ± 4, 257 ± 5 and 320 ± 9 $L_{CH_4}\,kg_{vs}^{-1}$ for I_{OM}, I_{CM} and I_R, respectively. The average methane concentration in the biogas generated by I_R was also higher (68 ± 7%) than that observed in the biogas generated by I_{OM} (64 ± 4%) and I_{CM} (58 ± 5%). Thus, I_R was used for the codigestion of S_{CM} and S_{CW} since it presented remarkably higher methane production.

The second set of BMP tests was conducted in order to evaluate the impact of different S_{CW}:S_{CM} ratios on AD mediated by I_R. The methane yields obtained using S_{CW} and S_{CM} separately were 12 ± 3 and 131 ± 7 $mL_{CH_4}\,g_{vs}^{-1}$, respectively. The BMP test using S_{CW} (S_{CW}:S_{CM} – 100:0) surprisingly showed very low methane production. However, chemical analyses demonstrated an accumulation of VFAs (data not shown) with pH decrease (down to 4.2) just a few days after the beginning of the test. Similar findings were reported by other authors (Ghaly, 1996; Malaspina et al., 1996) who observed acidification and, thus, methanisation inhibition during AD of CW. The combination of S_{CW} and S_{CM} resulted in higher methanogenic performances (Fig. 2). In fact, methane yield of the codigestion (S_{CW}:S_{CM}-50:50) improved to 320 ± 9 $mL_{CH_4}\,g_{vs}^{-1}$ that is 2.5 the value obtained by CM and 27 times the value obtained by CW when used alone.

Although the methane yield increased with S_{CW} (S_{CW}:S_{CM} ratios of 0:100 to 50:50), CH_4 production fell when the S_{CW} fraction was higher than 60% (Fig. 2). Therefore, the results demonstrate that codigestion seems much more robust with the increase of the S_{CM} fraction and there is a threshold below which the process tends to acidify the medium. In fact, acidification to pH values below 6.2 (value for S_{CW}:S_{CM} 70:30) was observed when the S_{CW} fraction was higher than 60%. On the contrary, the increase of the S_{CW} fraction from 0% to 60% greatly improved the methane yield as a result of the higher content of biodegradable organic matter of S_{CW} as also proposed by Kavacik and Topaloglu (2010).

Methane concentrations comprised between 54% and 66% were measured in the headspaces irrespective of the applied S_{CW}:S_{CM} ratios, demonstrating the low effect of the tested feed on biogas composition.

Maximum CH_4 production rate and complete CH_4 production depletion were measured after approximately 20 and 50 experimental days, respectively. Therefore, the bench-scale methanogenic stages were designed for HRT 20 d.

The third batch experiments were carried out to determine the optimal conditions of the acidogenic phase of the two-stage codigestion. The pH of the anaerobic liquor decreased just the day after the beginning of the trials by reaching values of 4.5–5.0 at the end of the experiments. The acidification, due to accumulation of VFAs, caused the inhibition of methanogenic activity coupled with hydrogen accumulation in the biogas (Chen et al., 2008). Total accumulation of VFAs up to 3700 $mg\,L^{-1}$ was observed in the

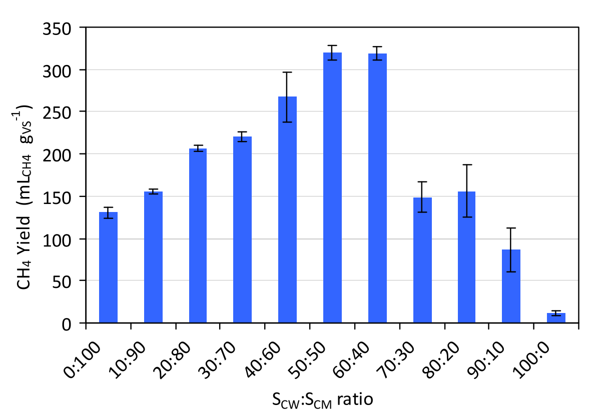

Fig. 2. Methane yields measured at different cheese whey: cattle manure volumetric ratio (S_{CW}:S_{CM}). Bars for SD.

Table 2
Main results of the bench scale reactors.

	pH		Removal (%)		[b]Productivity ($L\ L^{-1}\ d^{-1}$)			Composition (%)		Yield ($L\ CH_4\ kg_{VS}^{-1}$)
	Acidog.	Methan.	sCOD	Carbohydrate	CH_4	H_2	Biogas	CH_4	H_2	CH_4
R1	–	6.7 ± 0.5	71 ± 8	96	0.18 ± 0.04	–	0.38	48 ± 5	–	120
R2	4.9 ± 0.2	7.8 ± 0.4	80 ± 11	98	0.28 ± 0.05	0.1	0.42	[a]63 ± 7	[a]32 ± 4	233
R3	5.1 ± 0.3	7.5 ± 0.3	83 ± 6	98	0.31 ± 0.04	0.02	0.51	60 ± 6	2	258

[a] Values measured in the biogas from the headspace of the two separate stages.
[b] Values estimated on the volume of the entire systems.

acidogenic reactors. The main VFAs produced were acetic (concentration up to 1400 mg L^{-1}), butyric (up to 850 mg L^{-1}) and caproic (up to 730 mg L^{-1}) acids, whereas the other acids were detected at much lower concentrations (lower than 200 mg L^{-1}).

Biogas production yield was much higher under mesophilic conditions ($84 \pm 2\,mL_{H_2}\,g_{vs}^{-1}$) than under non-controlled temperature ($41 \pm 4\,mL_{H_2}\,g_{vs}^{-1}$). These results were comparable with those observed by fermentative batch tests on organic waste (Giordano et al., 2011) and by continuous mode on CW (Venetsaneas et al., 2009).

Maximum concentration of VFAs and biogas H_2 content (68 ± 4%) was observed within the first 5 experimental days; therefore, the HRT of 5 d was applied for the acidogenic stage under mesophilic conditions of the two-stage anaerobic reactors (Table 2).

3.3. Continuous codigestion experiments

The feed was periodically prepared by combining S_{CW} and S_{CM} at a volumetric ratio of 50% and stored at 4 °C. However, the feed showed slightly lower concentrations than the S_{CW} and S_{CM} average values probably due to the partial degradation of the easily biodegradable organic matter; the resulting feed characteristics are reported in Table 1.

pH occurring in the reaction media of the acidogenic and methanogenic stages of R2 and R3 were similar (Table 2), whereas R1 showed slightly acidic conditions. Therefore, the codigestion of CW and CM allowed the systems to maintain stable pH values at both stages.

Both two-stage processes seemed to show better sCOD removal compared with the one-stage reactor (Table 2); moreover, R3 seemed to reach slightly higher sCOD removal efficiency than R2 (although these differences were not statistically different). The average sCOD removal during the acidogenic stages of both two-stage reactors was found to be approximately 30%.

The total carbohydrate concentration in the effluents was consistently lower than 0.5 g L^{-1}, corresponding to removal yields that were always higher than 95% (Table 2).

Total VFAs accumulated in the acidogenic stage were 6.9 ± 0.15 g L^{-1} and 5.8 ± 1.68 g L^{-1} for R2 and R3, respectively; they were then decreased (to a total concentration of VFAs that was lower than 1.0 g L^{-1}) by acetotrophic methanogens in the methanogenic stage. On the contrary, total VFAs in R1 remained stable between 1.5 and 2.0 g L^{-1}. The main VFAs detected in the acidogenic stages were, acetic, caproic, butyric and propionic acids, while the effluents of the methanogenic stages were mostly composed of acetic acid.

The total concentration of VFAs measured in the present study was lower than those obtained by other studies treating CW and CM singularly, demonstrating that the codigestion of the two substrates greatly improved the degradation of VFAs. In fact, Ghaly (1996), using a two-stage reactor at HRT of 20 d, measured total VFA concentrations over 2.0 g L^{-1} and below 0.1 g L^{-1} for CW and CM, respectively, when used alone.

Methane production rate at steady state was generally stable in all three reactors. However, R2 and R3 showed methane production approximately 40% higher than R1 (Table 2). Moreover, although the biogas composition of the three reactors fell within the typical range for AD of agricultural waste (e.g. Comino et al., 2012), the two-stage systems also performed better than the one-stage process in terms of methane content (Table 2). It is of note that significant H_2 amounts were collected from the acidogenic stage of R2 (Table 2) due to the complete physical separation of the two stages, whereas the methane content was always below 5%.

The methane yield and the methane concentration in the biogas were also higher in the two-stage reactors than in the one-stage reactor (Table 2). However, it is important to specify that the methane percentage of 63% detected in R2 was related to the methanogenic stage, whereas the CH_4 percentage (60%) of R3 was measured over both stages.

The results of the continuous experiments (Table 2) showed lower yields than those obtained in batch conditions (Fig. 2). However, the maximum methane yield of $320 \pm 9\,mL_{CH_4}\,g_{vs}^{-1}$ achieved in batch conditions is related to "ultimate" biogas production (i.e. for complete substrate methanisation) that is obtained with a much longer test duration (50 d) than the HRT of the continuous experiments. Nevertheless, the yields obtained in this study are mostly in agreement with data recently reviewed by Esposito et al. (2012) and Nasir et al. (2012).

The results, therefore, demonstrate the much higher efficiency of the two-stage systems than the one-stage one treating CW and CM in codigestion.

4. Conclusions

The results demonstrate that the AD of CW and CM at 50% volumetric ratio provides higher biomethanisation yields than when the two wastes undergo the same process individually.

Moreover, the study demonstrates the much higher efficiency of the two-stage system rather than the one-stage system treating CW and CM in codigestion.

The concentric two-stage reactor obtained a slightly higher methane yield that could be explained by better use of the hydrogen produced in the acidogenic phase, which, with the lower footprint, could represent an improvement of AD for agroindustrial waste codigestion.

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