Bioresource Technology 128 (2013) 612–618

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Co-digestion of livestock effluents, energy crops and agro-waste: Feeding and process optimization in mesophilic and thermophilic conditions

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HIGHLIGHTS

- ▶ The co-digestion of several substrates coming from agricultural sector was studied.
- ▶ We performed both batch and continuous trials under different operating conditions.
- ▶ The partial substitution of energy crops with agro-waste increases the biogas yields.
- ▶ The greatest biogas yields were reached in thermophilic conditions.

ARTICLE INFO

Article history:
Received 26 July 2012
Received in revised form 1 November 2012
Accepted 1 November 2012
Available online 10 November 2012

Keywords:
Anaerobic co-digestion
Agro-waste
Energy crops
Biochemical methane potential
Free ammonia

ABSTRACT

In this study the optimization of the biogas yield from anaerobic co-digestion of manures and energy crops was carried out using four pilot scale CSTRs under different operating conditions. The effect on biogas yield of the partial substitution of energy crops with agro-waste was also investigated. For each substrate used during the continuous trials, BMP batch assays were also carried out to verify the maximum methane yield theoretically obtainable. Continuous operation results indicated that the co-digestion of manures, energy crops and agro-waste was viable at all operating conditions tested, with the greatest specific gas production of 0.54 $m^3/kg\ VS_{fed}$ at an organic load rate of 2 kg $TVS/m^3_r d$ consisting of 50% manure, 25% energy crops and 25% agro-waste on VS basis. No significant differences were observed between high and low loaded reactors suggesting the possibility of either improving the OLR in existing anaerobic reactors or reducing the design volumes of new reactors.

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1. Introduction

Anaerobic digestion is one of the most common practices for the management of livestock manure because of the renewable biogas energy which can be obtained. A lot of biogas plants have been built as a consequence of European incentives for renewable energies (Lindorfer et al., 2008a; Bolzonella et al., 2011). Livestock effluents are characterized by a low carbon to nitrogen ratio (C/N) due to relatively high concentration of ammonia, limiting the biogas yields (Procházka et al., 2012). A way to decrease the risk of ammonia inhibition is the co-digestion of livestock effluents with energy crops. The addition of energy crops (mainly maize silage) with high carbon content balances the C/N ratio of the feedstock (Wang et al., 2012) increasing the energy balance of the reactors (Amon et al., 2007a) and optimizing the economic revenue derived from the European subsidies received (Bolzonella et al., 2011). However, energy crops need water, energy and fertilizers (Hanegraaf et al., 1998), decreasing, at the same time, the amount of arable land surface available for food crop production with a subsequent increase in the cost of food crops. Therefore, from a Life Cycle Assessment (LCA) point of view, these substrates are penalized (see Annex V.A in Directive 2009/28/EC on renewable energies). In addition, several environmental factors negatively influence their biodegradability (Amon et al., 2007b),

Abbreviations: AD, anaerobic digestion; AV, average value; COD, chemical oxygen demand; CSTRs, continuous stirred tank reactors; GPR, gas production rate; HRT, hydraulic retention time; *K*h, hydrolysis constant; NH_3, free ammonia; N-NH_4^+, total ammonia; OLR, organic load rate; P, phosphorus; PA, partial alkalinity; $R1_{H37}$, high loaded mesophilic reactor; $R2_{L37}$, low loaded mesophilic reactor; $R3_{L55}$, low loaded thermophilic reactor; $R4_{H55}$, high loaded thermophilic reactor; S.D, standard deviation; SGP, specific gas production; T, temperature; TA, total alkalinity; TKN, total Kjiendhal nitrogen; TS, total solids; TVS, total volatile solids; V, volume; VFA, volatile fatty acids; w.w, wet weight; d.w, dry weight.

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http://dx.doi.org/10.1016/j.biortech.2012.11.002

making energy crops an unfavorable feedstock. A cost effective way to facilitate future development of the agro-economy can be the substitution of energy crops with several kinds of agro-waste in AD plant feedstock (Schievano et al., 2009). Because of its abundance, agro-waste can be considered an ideal substrate to substitute energy crops in the co-digestion process. The anaerobic co-digestion of manure and agro-waste is already widely used in Europe (Weiland, 2010).

The performance in terms of biogas production and digestate quality depends on several parameters such us temperature, OLR, HRT and feedstock composition.

The effect of temperature on process efficiency has been investigated through different studies, although studies of both mesophilic and thermophilic digesters have conflicting results (Ward et al., 2008). Lindorfer et al. (2008b) showed that there is no general temperature optima in anaerobic digestion and process performance depends on many other parameters such as nitrogen load, hygienic requirements and plant design. Cavinato et al. (2010) showed that biogas production from the co-digestion of cattle manure and other organic waste can be increased when operating at proper thermophilic conditions (55 °C) and also a general improvement in digester behavior was clear considering the stability parameters. On the other hand, a decrease in thermophilic reactor performance may occur if free ammonia concentration exceeds approx. 0.7 g-N/l (Amon et al., 2007a,b; Angelidaky and Aharing, 1994; Cavinato et al., 2012).

As far as the organic load and retention time are concerned, in northern Italy most AD farm plants operate in the range of 1–3 kg TVS/m^3_rd with retention time always greater than 40 days (Bolzonella et al., 2011).

Several authors have showed that it is possible to increase the OLR of conventional biogas plants which treat both manure and energy crops but to avoid atmospheric emissions, the effluent storage of high loaded processes has to be integrated into the gas-tight system of the digesters (Lindorfer et al., 2008a; Comino et al., 2010; Menardo et al., 2011).

In the present paper we studied the optimization of anaerobic digestion of livestock effluents and co-treatment with energy crops under different operating conditions. The effect on biogas yields of the partial substitution of energy crops with agro-waste was also investigated. In particular four parallel pilot scale continuous stirred tank reactors were operated under different temperature conditions, organic load rate and hydraulic retention times.

2. Methods

2.1. Substrates and BMP assays

The substrates used for this experimentation, namely cattle slurry, cow manure, maize silage, triticale and agro-waste (potatoes and onions) came from an AD plant treating livestock effluents, energy crops and other organic co-substrates. Table 1 shows the average values found for the characterization of each substrate.

Cattle slurry and cow manure showed a TS content of 31% and 9% respectively, with a high volatile fraction (80% on TS). The COD/TS ratio was in the range of 0.83–0.92, indicating a high level of oxidation as expected for a "digested" substrate. Energy crops and agro-waste showed a higher VS concentration (more than 90%) with a COD/TS ratio greater than 1. As expected, livestock effluents showed a high nitrogen content: in the case of cattle slurry ammonia concentration was more than 50%, reaching concentrations as high as 2 g/L. Also, phosphorous showed concentrations in the range of 6–8 g/kg (as P).

On the other hand, energy crops and agro-waste showed lower concentrations of nutrients if compared with livestock effluents: in particular, energy crops showed N and P concentrations at around 13–17 g N/kg TS and 1.4–2.5 g P/kg TS respectively, while the concentrations of nutrients found for agro-waste were in the range of 23–27 g N/kg TS and 2–3 g P/kg TS, respectively.

For each substrate, BMP assays were performed both in mesophilic and thermophilic conditions for at least 30 days in accordance with the method described by Angelidaki et al. (2009). Each batch of assays was carried out in triplicate using 1 liter closed vessels, inoculated with 500 mL of digested sludge drawn from the farm-scale anaerobic digestion plant treating livestock effluents and other co-substrates. Before starting the assays, the sludge was maintained for 2 weeks at proper temperatures (37° and 55 °C) in order to acclimatize the microorganisms to the new thermal conditions.

2.2. Reactors and experimental design

Four parallel pilot scale CSTRs were used to study the combined influence of temperature, OLR, HRT and feedstock composition. Heating of the reactors was provided by an external heater and the reactor temperature control was carried out through resistance temperature detectors. The reactors were fed semi-continuously, 3 times per day. Table 2 shows the experimental operational conditions and feedstock composition used in the different runs adopted in this study.

The working volume of each reactor was 0.23 m^3. $R1_{(H37)}$ and $R2_{(L37)}$ and were maintained at 37 °C whereas $R3_{(L55)}$ and $R4_{(H55)}$ were operated at 55 °C. High (4 kg TVS/m^3_rd) and low (2 kg TVS/m^3_rd) organic loading rates were compared using two different hydraulic retention times, 30 days and 60 days for $R1_{(H37)}$/$R4_{(H55)}$ and $R2_{(L37)}$/$R3_{(L55)}$ respectively. In order to study the influence of feedstock composition, three runs were carried out (Table 2). RUN I was characterized by a feedstock consisting of cattle slurry and cow manure (livestock effluents) with a ratio 1.5:1 on a wet weight base, a typical situation for dairy farms. During RUN II, 50% of the OLR included the addition of energy crops (triticale and maize silage) and the remaining 50% consisted of livestock effluents on a VS basis, whereas during RUN III the OLR included 50% livestock effluents, 25% energy crops and 25% agro-waste respectively, on a VS basis. In order to control the TS concentration of feedstock, during RUN II and RUN III, part of the effluent liquid fraction of each reactor was recirculated in the influent feedstock mixing tank. The reactors were operated for 390 days in all.

Table 1
Average characteristics and standard deviation for the different substrates.

A.V ± S.D		TS (g/kg)	TVS/TS (%)	COD (g/kg TS)	TKN (g/kg TS)	P (g/kg TS)
LE	Cattle slurry	94 ± 45	79 ± 2	907 ± 245	38 ± 2	8 ± 2
	Cow manure	310 ± 35	80 ± 3	835 ± 201	49 ± 3	6 ± 0.5
ECs	Triticale	251 ± 51	90 ± 1	1050 ± 95	17 ± 1	1.4 ± 0.02
	Maize silage	347 ± 54	96 ± 1	1100 ± 112	13 ± 3	2.5 ± 0.02
AW	Onion	107 ± 15	93 ± 0.5	940 ± 85	27 ± 1	3 ± 0.02
	Potato	177 ± 28	94 ± 0.5	1000 ± 89	23 ± 1	2 ± 0.02

Table 2
Operational conditions and feedstock composition.

Operating conditions	Reactors			
	$R1_{(H37)}$	$R2_{(L37)}$	$R3_{(L55)}$	$R4_{(H55)}$
V (m^3)	0.23	0.23	0.23	0.23
T (°C)	37	37	55	55
HRT (d)	30	60	60	30
OLR (kg TVS/m^3_rd)	4	2	2	4
Run time	Feed composition*			
RUN I	100% LE			
RUN II	50% LE + 50% ECs			
RUN III	50% LE + 25% ECs + 25% AW			

* On VS basis.

2.3. Inoculum

The inoculum sludge was obtained from a biogas plant previously described in Cavinato et al. (2010). It treats a mixture of livestock effluents, energy crops (triticale, maize silage) and other food and agro-waste (fruit, vegetables, bread, grass, oil etc.), operating at an average temperature of 41 °C and an OLR of 2.1 kg TVS/m^3_rd for a retention time of some 60 days.

The inoculum sludge was characterized by a TS content of 5% (65% VS), a pH of 8.6, a VFA content, around 1 g COD/L, mainly constituted by acetic acid, and an alkalinity of 15,782 mg $CaCO_3$/L on average. Ammonia concentration was 3790 mg N/L on average.

In order to support microorganism adaption at new thermal conditions without undergoing the stress of organic loading during the mesophilic–thermophilic passage, the temperature of all reactors were changed into thermophilic conditions in a very short time (a couple of days) while feeding was stopped, using a protocol already reported in previous papers by the authors (Bolzonella et al., 2003a).

2.4. Analytical methods

The effluent of each reactor was monitored 3 times a week for the parameters total solids (TS) and volatile solids (VS), chemical oxygen demand (COD), total Kjieldhal nitrogen (TKN), total phosphorus (P) and stability parameters (pH, alkalinity, ammonia and VFA content), all in accordance with Standard Methods (APHA, 1998). The VFA content was monitored using a gas chromatograph (Carlo Erba instruments) with hydrogen as gas carrier, equipped with a Fused Silica Capillary Column (Supelco NUKOL™, 5 m × 0.53 mm × 0.5 mm film thickness) and with a flame ionization detector (200 °C). The analyzed samples were centrifuged and filtered with a 0.45 μm membrane. Gas production was monitored by a gas flow meter (Ritter Company, drum-type wet-test volumetric gas meters), while the biogas composition (CO_2–CH_4) was defined by a portable infrared gas analyzer (Geotechnical Instrument, model GA2000). Methane content in the BMP trials was measured by a gas-chromatograph (GC Agilent Technology 6890N) equipped with the column HP-PLOT MOLESIEVE, 30 m × 0.53 mm ID × 25 μm film, using a thermal conductivity detector and argon as gas carrier.

3. Results and discussion

3.1. BMP assays

The ultimate biogas obtainable from the substrates used in this experimentation was determined through BMP trials. Several substrates used to feed the experimental rigs were used to measure the specific yields in terms of biogas (m^3_{biogas}/kg TVS) and methane (m^3 CH_4/kg TVS) production. Moreover, using the first part of the experimental curve built for the determination of the ultimate methane production of different substrates (data not shown) and the methodology given in Angelidaki et al. (2009), it was possible to determinate the value of the first order hydrolysis constant (*K*h, 1/d). Table 3 shows the experimental data obtained in both mesophilic and thermophilic conditions. The results of BMP assays show clearly that livestock effluents are unfavorable feedstock because of low methane yield (from 0.1 to 0.3 m^3 CH_4/kg TVS_{fed}). On the other hand, livestock effluents are an important feedstock to gain nutrients and buffer capacity due to the high nitrogen content (Table 1). The most significant mechanism of buffering is based on ammonium nitrogen, CO_2 and VFA equilibrium. Carbonate equilibrium can be shifted by VFA with lower pKa than the pKa of CO_2 towards free CO_2 which does not affect pH value. VFA is then neutralized with ammonium nitrogen (Procházka et al., 2012). The thermophilic batch assays of livestock effluents showed that specific methane yields were around 20% higher than under mesophilic conditions (except for the cow manure). As far as the results from the energy crops were concerned, the methane mesophilic yields for both triticale and maize were 0.4 m^3 CH_4 per kg TVS_{fed}, while under thermophilic conditions they were 0.45 and 0.55 m^3 CH_4 per VS kg fed respectively. Finally, ultimate methane potential reached by agro residues (onions and potatoes) were in the range of 0.42–0.46 and 0.48–0.55 kg m^3CH_4 per kg VS fed for mesophilic and thermophilic assays respectively. All these results are in agreement with literature data (Pabon Pereira et al., 2009, Labatut et al., 2009). Looking at the first order hydrolysis constant reported in Table 3, the highest values were observed for agro-waste, the most biodegradable substrates, and in thermophilic conditions confirming the dependence of the first-order hydrolysis rate on temperature (Vavilin et al., 2008), the only exception being the cattle slurry. In general, from the batch tests it can be concluded that thermophilic range (55 °C) is more suitable than mesophilic range (37 °C) in terms of methane yield for most of the substrates given in this study with a higher reaction rate of the hydrolysis step (some 10% more than mesophilic conditions), confirmed by the experimental values of *K*h obtained.

3.2. Transient and steady state conditions

During the start-up phase, in two reactors ($R1_{H37}$, $R4_{H55}$) the OLR was increased from 2 kg TVS/m^3_rd to 4 kg TVS/m^3_rd, whereas

Table 3
Results from the batch trials in mesophilic and thermophilic conditions.

	Mesophilic			Thermophilic		
	SGP (m^3_{biogas}/kg TVS)	SMP (m^3 CH_4/kg TVS)	*K*h d^{-1}	SGP (m^3_{biogas}/kg TVS)	SMP (m^3CH_4/kg TVS)	*K*h d^{-1}
Cattle slurry	0.18	0.11	0.13	0.18	0.13	0.12
Cow manure	0.22	0.12	0.12	0.23	0.12	0.13
Triticale	0.76	0.40	0.21	0.91	0.45	0.23
Maize silage	0.73	0.40	0.21	0.82	0.55	0.23
Onion	0.92	0.46	0.34	1.05	0.55	0.37
Potato	0.83	0.42	0.26	0.92	0.48	0.30

it was maintained at 2 kg TVS/m^3_rd throughout the entire experimental period for the other two reactors ($R2_{L37}$ and $R3_{L55}$). Time profiles of pH, total alkalinity (TA), total ammonia (N-NH_4^+) and macronutrient contents (COD, TKN and P) showed substantial stability from the very beginning for the mesophilic tests. On the contrary, parameters TS, VFA, partial alkalinity (PA), biogas production and biogas composition showed a typical transient condition (Bolzonella et al., 2003b). After loading, the VFA concentration reached a peak in each reactor, more evident at higher OLRs, and then it assumed a downward trend mainly due to acetate consumption (Fig. 1). With specific reference to VFA profiles, mesophilic reactors reached a steady condition after 45 days and 30 days for $R1_{H37}$ and $R2_{L37}$, respectively. The only evidence of instability was observed in $R1_{H37}$ where VFA concentration increased, in accordance with an increase of OLR (75 days); however, this situation was completely recovered after 2 weeks. Because of higher values of the kinetic coefficients for the first-order rate of hydrolysis, VFA content of thermophilic reactors was higher than that of the mesophilic reactors, confirming the data obtained in batch assays. Moreover, previous works have shown that the main methanogenic pathway which occurs in thermophilic conditions is performed by hydrogen-trophic bacteria, while the acetoclastic bacteria are predominant in mesophilic conditions (Vavlin, 2010). This could explain the faster acetate consumption observed in both $R1_{H37}$ and $R2_{L37}$. During RUN I an increase of VFA content was observed in both thermophilic reactors with a concomitant methane reduction into biogas. After 120 days, the OLR for both $R3_{L55}$ and $R4_{H55}$ was gradually lowered to 1.4 and 2.8 kg TVS/m^3_rd respectively, via partial substitution of manure with the liquid fraction coming from the effluent. However, both reactors showed unstable behavior resulting in failure: high VFA content (up to 7000 mg COD/L) and breakdown of specific gas production (Fig. 2). Therefore, the feeding operations were stopped for 2 weeks, where a VFA reduction was observed. Because of high nitrogen content of livestock effluent, the treatment of these substrates produces an effluent with a high concentration of total ammonia (Table 4): from 3245 ($R4_{H55}$) to 3886 mg/L ($R2_{L37}$). Considering the different values of both pH and temperature, the unionized ammonia (NH_3) was some 1000, 1440, 1740 and 1800 mg/L for $R1_{H37}$, $R2_{L37}$, $R3_{L55}$ and $R4_{H55}$, respectively. Several authors have shown that under high ammonia concentrations hydrogen-trophic methanogenesis is the principle route to methane formation (Banks et al., 2012). Wiegant and Zeeman (1986) explained the mechanism of ammonia inhibition in thermophilic anaerobic digesters in which the NH_3 concentration plays a role as a strong inhibitor of methane generation from H_2 and CO_2. The independence of the specific growth rate of acetate-consuming methanogens from NH_3 leads to only a minor effect on the methane production from acetate. Fig. 3 shows the effluent VFA concentration in relation to NH_3 concentration in the thermophilic reactors. The data reported are in agreement with the ones reported by Angelidaky and Aharing (1994). In particular, the effect of NH_3 is to inhibit propionate breakdown. In the light of this observation, during the last part of RUN I when a failure of biogas production occurred in the thermophilic reactors, the propionic acid was 44% ($R3_{L55}$) and 35% ($R4_{H55}$) of total VFA, confirming the role of propionic acid as a good indicator in preventing an imbalance in the anaerobic trophic chain (Nielsen et al., 2007). It can be remarked that thermophilic anaerobic digestion of livestock effluents (cattle slurry and cow manure) led to higher free ammo-

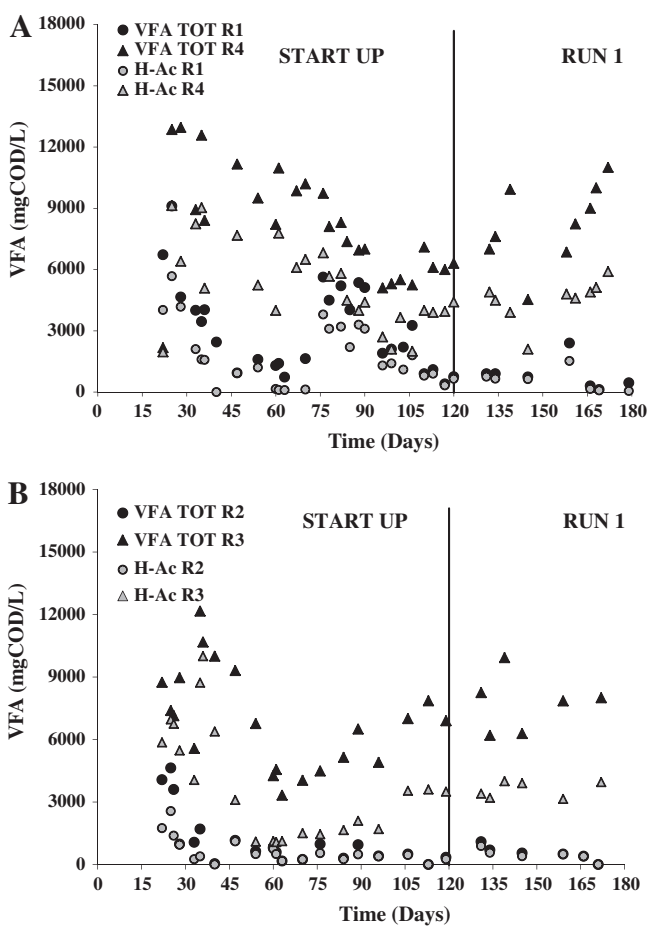

Fig. 1. Time profile of VFA during the start-up phase and RUN I: (A) "high" loaded reactors and (B) "low" loaded reactors. H-Ac = acetic acid.

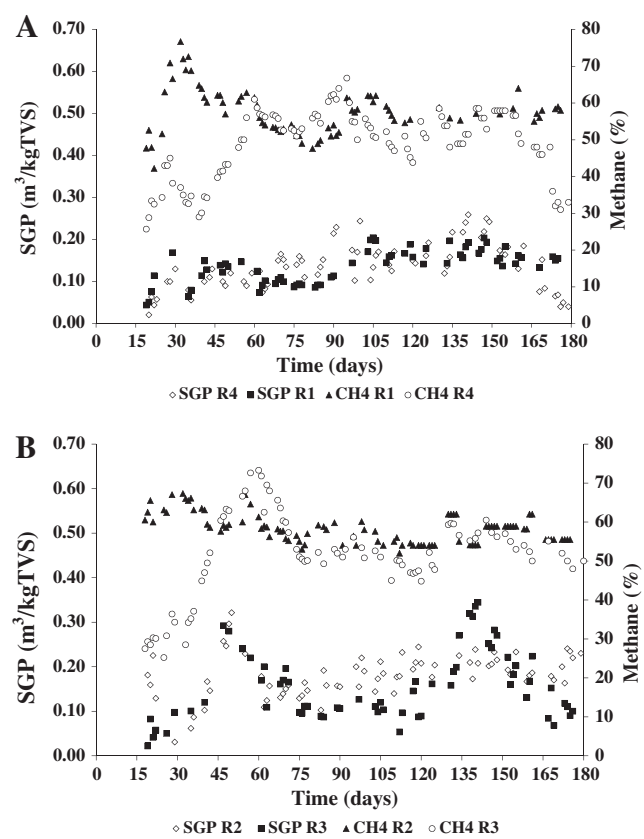

Fig. 2. Time profile of SGP and gas composition during start-up phase and RUN I: (A) "high" loaded reactors and (B) "low" loaded reactors.

Table 4
Average characteristics and standard deviations of the digesters effluents.

A.V ± S.D		RUN I				RUN II				RUN III			
		$R1_{H37}$	$R2_{L37}$	$R3_{L55}$	$R4_{H55}$	$R1_{H37}$	$R2_{L37}$	$R3_{L55}$	$R4_{H55}$	$R1_{H37}$	$R2_{L37}$	$R3_{L55}$	$R4_{H55}$
Effluent characteristics	Unit												
pH	–	8.6 ± 0.3	8.6 ± 0.2	8.8 ± 0.2	8.6 ± 0.06	8.2 ± 0.2	8.3 ± 0.1	8.2 ± 0.1	8.1 ± 0.2	8.3 ± 0.2	8.5 ± 0.1	8.5 ± 0.1	8.5 ± 0.1
TA (pH4)	mg $CaCO_3$/L	10820 ± 1395	13468 ± 1774	11103 ± 565	11791 ± 823	20262 ± 1245	21671 ± 958	15353 ± 785	16253 ± 885	21152 ± 924	19800 ± 445	17320 ± 657	18320 ± 774
N-NH_4^+	mg N/L	3513 ± 301	3886 ± 574	3340 ± 270	3245 ± 201	2626 ± 354	2750 ± 114	2900 ± 254	2798 ± 224	2600 ± 125	2775 ± 241	2100 ± 321	1450 ± 425
VFA	mg COD/L	846 ± 42	605 ± 280	7752 ± 1385	8239 ± 1992	1616 ± 251	403 ± 100	2503 ± 380	2989 ± 210	66 ± 115	115 ± 34	172 ± 89	238 ± 114
TS	g TS/kg w.w	107 ± 5	99 ± 5	75 ± 12	83 ± 7	92 ± 0.4	81 ± 0.2	81 ± 0.2	87 ± 0.2	89 ± 0.3	85 ± 0.3	81 ± 0.2	90 ± 0.3
TVS	%	76 ± 4	71 ± 0.5	70 ± 0.7	72 ± 0.7	72 ± 0.1	71 ± 0.2	74 ± 0.2	72 ± 0.1	74 ± 0.2	73 ± 0.1	72 ± 0.1	72 ± 0.1
COD	g COD/kg d.w	797 ± 29	775 ± 63	762 ± 80	827 ± 85	850 ± 112	884 ± 98	868 ± 85	829 ± 85	743 ± 112	791 ± 170	810 ± 103	730 ± 111
TKN	g N/kg d.w	28 ± 4	28 ± 3	33 ± 4	30 ± 3	14 ± 3	12 ± 2	11 ± 2	12 ± 1	30 ± 2	29 ± 3	34 ± 3	33 ± 1
P	g P/kg d.w	4.6 ± 1	5 ± 0.5	5.2 ± 1.4	5.4 ± 1.2	5 ± 1	5 ± 1	5 ± 1	5 ± 1	6.4 ± 1	6.6 ± 1	6 ± 1	4 ± 1
Biogas yields													
GPR	m^3/m^3.d	0.79 ± 0.07	0.49 ± 0.04	Failure	Failure	1.3 ± 0.05	0.74 ± 0.05	0.79 ± 0.05	1.3 ± 0.04	2 ± 0.01	0.98 ± 0.01	1.13 ± 0.02	2.17 ± 0.02
SGP	m^3/kg TVS_f	0.18 ± 0.02	0.23 ± 0.02	Failure	Failure	0.32 ± 0.01	0.37 ± 0.01	0.40 ± 0.01	0.41 ± 0.02	0.48 ± 0.01	0.47 ± 0.02	0.54 ± 0.01	0.52 ± 0.01
CH_4	%	57 ± 3	57 ± 3	–	–	53 ± 1	56.2 ± 1	55.4 ± 1	54.6 ± 1	52 ± 1	54 ± 1	53 ± 1	54 ± 1
CO_2	%	42 ± 3	42 ± 3	–	–	45 ± 1	43.2 ± 1	44.4 ± 1	44.5 ± 1	48 ± 1	46 ± 1	47 ± 1	46 ± 1

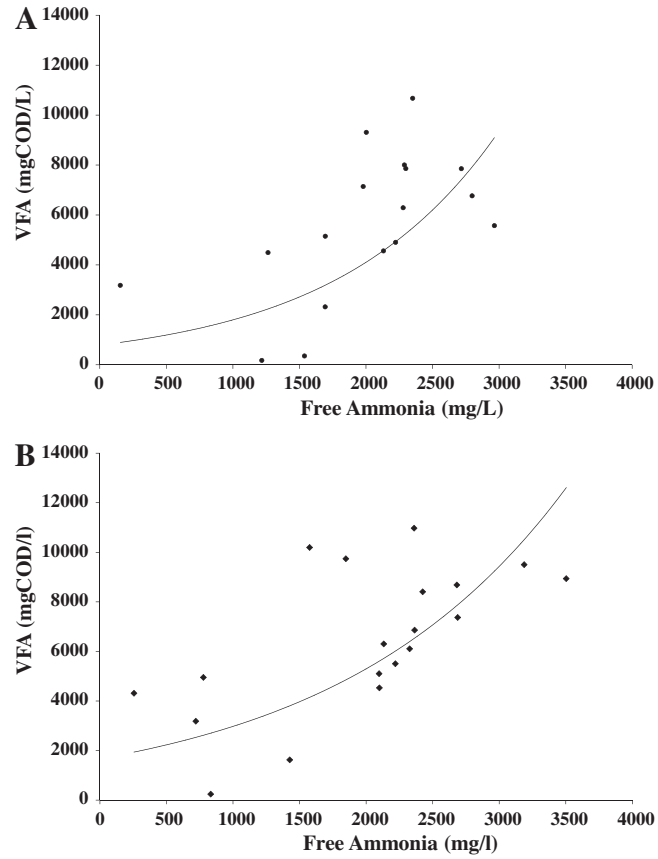

Fig. 3. Effluent VFA concentration as a function of the free ammonia content: (A) thermophilic "low" loaded reactor and (B) thermophilic "high" loaded reactor.

nia contents (NH_3) in the effluent that in turn caused VFA accumulation and consequent failure of the reactors.

The mesophilic reactors during RUN I, $R1_{H37}$ and $R2_{L37}$ showed the typical performance of biomass treatment with a high degree of oxidation, reported by previous works (Demirer and Chen, 2008; Capela et al., 2008; Buendía et al., 2009) giving an SGP of 0.18 and 0.23 m^3/kg TVS for $R1_{H37}$ and $R2_{L37}$ respectively (more or less similar to the maximum biogas yields achievable in mesophilic conditions, 0.21 m^3_{biogas}/kg TVS), with a low VFA concentration (less than 1 g COD/L) and high alkalinity (some 10,800 and 13,500 mg $CaCO_3$/L for $R1_{H37}$ and $R2_{L37}$, respectively).

As mentioned before, most AD farm plants treat livestock effluents and energy crops, especially maize silage (Weiland, 2010), therefore, in the second part of the study, we tested the co-digestion of livestock effluent together with energy crops. Cornell and Banks (2012) reported that the addition of equal amounts of volatile solids of maize to slurry leads to a significant increase in the volumetric methane yield. Therefore, in order to study the optimum operating conditions for the co-treatment of livestock effluents with energy crops, the continuous experimental trial was carried out substituting 50% of the organic load on VS basis with energy crops (RUN II). The major differences (Table 4) during RUN II compared with the digestion of livestock effluents only, were:

- An higher total alkalinity for both mesophilic (more than 85% and 60% for $R1_{H37}$ and $R2_{L37}$ respectively) and thermophilic (around 38%) reactors.
- A lower VFA concentration in thermophilic reactors (2503 and 2989 mg COD/L for $R3_{L55}$ and $R4_{H55}$ respectively). Doubled VFA content (from 846 to 1616 mg COD/L) was observed in $R1_{H37}$, while $R2_{L37}$ maintained more or less the same concentration (403 mgCOD/L).

® An increase in biogas yield. The highest SGP was reached by $R4_{H55}$ (0.41 m^3_{biogas}/kg TVS), 28% more than $R1_{H37}$ (0.32 m^3_{biogas}/kg TVS), whereas $R3_{L55}$ reached a value (0.40 m^3_{biogas}/kg TVS) of 8% more than R2L37 (0.37 m^3_{biogas}/kg TVS).

The biogas yield obtained during the co-treatment of livestock effluent and energy crops under mesophilic conditions demonstrated a 78% and 61% increase for $R1_{H37}$ and $R2_{L37}$ respectively. As reported in Table 4 the co-digestion of livestock effluents with energy crops showed the greatest biogas yields in thermophilic conditions at 4 kg TVS/m^3_rd OLR and 30 d retention time. Under these operating conditions the reactor ($R4_{H55}$) showed very stable behavior confirmed by the high alkalinity value (some 16.2 g $CaCO_3$/L) allowing the pH to buffer around 8.1. The maximum gas production rate obtained was 1.3 m^3_{biogas}/m^3_rd with a CH_4 content of 55%. The higher SGP obtained during RUN II is due to the presence of a highly biodegradable compound contained in the volatile solids fraction of energy crops, as reported by Lehtomaki et al. (2007). Furthermore during RUN II, the partial substitution of livestock effluents with energy crops led to a lower nitrogen load. Consequently, all reactors showed a decrease of the N-NH_4^+ level in their effluents (some 2800 mg/L), which in turn resulted in a free ammonia concentrations lower than the critical values observed during RUN I (some 500, 450, 725 and 730 mg/L for $R1_{H37}$, $R2_{L37}$, $R3_{L55}$ and $R4_{H55}$ respectively).

It is confirmed that energy crops used as co-substrate with livestock effluents increase the efficiency of the reactors under both mesophilic and thermophilic conditions. However, not all reactors achieve the maximum biogas yields predicted on the biochemical methane potential basis. These gaps ranged from 20% ($R2_{L37}$, $R3_{L55}$ and $R4_{H55}$) to 30% ($R1_{H37}$) less than theoretical yields suggesting the need for a further optimization strategy to improve the digestion of slowly degradable fraction (mainly lignin) of the maize and cattle slurry (Lehtomaki et al., 2007).

In order to investigate the possibility of substituting the energy crops, which, on a long term perspective, are not sustainable because of their requirements for power, water and land, with a more sustainable agro-waste, half of the organic load consisting of energy crops applied during RUN III was replaced with a mixture of vegetable waste. Therefore during the last part of experimentation (RUN III), the OLR on VS base was made up of 50% of livestock effluents, 25% of energy crops and 25% of agro-waste. The choice of this substrate ratio reflects the situation observed in several AD farm plants which co-treat manures, energy crops and agro- waste around the Europe (Lehtomäki, 2006; Bolzonella et al., 2011; Lindorfer et al., 2008a).

During the last experimental run the stability of all reactors was not affected (Table 4). Each reactor showed a stable time profile of pH, (above 8) with a high buffer capacity (above 17 g $CaCO_3$/L). The good biological activity of the anaerobic trophic chain under all operating conditions during RUN III was confirmed by low volatile fatty acids concentration, always below 500 mg COD/L. A further increase in biogas production compared with RUN II was observed. The mesophilic reactors showed similar specific biogas production (0.47–0.48 m^3_{biogas}/kg TVS), 50% and 27% more than the values obtained during RUN II for the high and low loaded reactor, respectively. Better biogas production was achieved by thermophilic digesters. The SGP of the thermophilic high loaded reactor ($R4_{H55}$) was 0.52 m^3_{biogas}/kg TVS_{fed} (27% more than RUN II), whereas the greatest biogas yield (0.54 m^3_{biogas}/kg TVS corresponding to 35% more than RUN II) was reached by $R3_{L55}$, operating at 2 kg TVS/m^3_rd OLR and 30 d HRT. Under this condition the reactor showed a stable behavior with an high total alcalinity (17,320 mg $CaCo_3$/L) and a pH value over 8. The difference between the total and partial alcalinity showed a low value over the entire period, indicating a good balance of the anaerobic trophic chain,

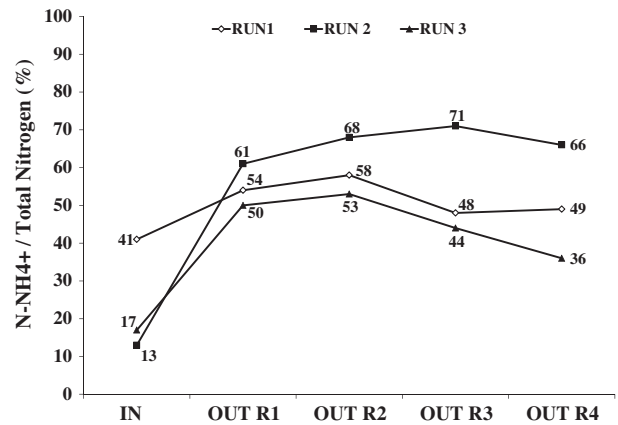

Fig. 4. Fraction of ammonia nitrogen (N-NH_4^+) with respect to the total nitrogen concentration in the influent and effluent streams.

confirmed by a low VFA concentration (172 mg COD/L). The biogas production rate resulted in 1.13 m^3_{biogas}/m^3_rd witn a 53% CH_4 content. The effluent of R3L55 showed a TS concentration around 8% of total wet weight with a 48% of total solid removal.

Under all operating conditions the biogas yields obtained in co-digestion of livestock effluents (cow slurry and cattle manure with a ratio 1.5:1 on a wet weight base) with energy crops and agro-waste were very close (above 94% of the maximum yield) to the predicted value (0.50 and 0.54 m^3_{biogas}/kg TVS for mesophilic and thermophilic reactors, respectively) determined in batch assays (BMP). This suggests that the slowly degradable fraction contained in agro-waste is lower than in energy crops: in fact, the comparison of the *K*h values, clearly shows that agro-waste are more easily degradable than energy crops.

Finally, the mass balance analysis carried out on nitrogen around the system (Fig. 4) showed that the degree of conversion of particulate organic nitrogen (proteins) into ammonia nitrogen ("ammonification") was lower in both thermal conditions during RUN I, confirming the low degradability of livestock effluents. Considering the treatment of livestock effluents, only higher ammonification occurred in the mesophilic low loaded reactor ($R2_{L37}$) where the fraction of ammonia nitrogen compared with total nitrogen increased from 41% in the influent to 58% in the effluent, 5% more than in the mesophilic high loaded reactor (operated with lower HRT). The thermophilic reactors showed low efficiencies and their failure was confirmed by low ammonification (7% and 8% for $R3_{L55}$ and $R4_{H55}$ respectively) demonstrating low capability in VS destruction. A higher degree of ammonification was obtained in the second run, due to the presence of highly biodegradable energy crops. In particular, the ammonia nitrogen fraction in the effluent streams was 61% and 66% of total nitrogen for $R1_{H37}$ and $R4_{H55}$ respectively. High values were obtained in low loaded reactors also (68% for $R2_{L37}$ and 71% for $R3_{L55}$). A smaller amount of ammonia nitrogen was finally reported in the third run where the co-treatment of livestock effluents with energy crops and agro-waste was performed. In this case, the degree of ammonification was 33% ($R1_{H37}$) and 36% ($R2_{L37}$) for mesophilic reactors and 27% ($R3_{L55}$) and 19% ($R4_{H55}$) for thermophilic reactors respectively. With specific reference to the last run, where the highest specific gas production was also obtained, thermophilic anaerobic digestion was more suitable compared to mesophilic conditions as far as nitrogen handling in downstream flows is concerned.

4. Conclusions

The work showed clearly that co-treatment of livestock effluents with energy crops and agro-waste can be applied. The

addition of agro-waste can be considered a proper choice for both mesophilic and thermophilic conditions. The results showed that the thermophilic process was able to cope with these kinds of substrates. Moreover, no significant differences were observed in terms of process stability between "high" and "low" OLR suggesting that it is possible either to increase the organic loading rate in existing anaerobic reactors or reduce the design volumes for new reactors.

Acknowledgement

This study was financially supported by the RiduCaReflui project coordinated by Veneto Agricoltura Spa and funded by the Veneto Region.

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