Adv. Hort. Sci., 2019 33(3): 333-344 DOI: 10.13128/ahs-23633

AHS
Advances in Horticultural Science

Preliminary screening of agricultural feedstocks for anaerobic digestion

E. Palchetti [1 (*)], A. Calamai [1], L. Verdi [1], A. Masoni [1], L. Marini [1], D. Chiaramonti [2]

[1] *Dipartimento di Scienze e Tecnologie Agrarie, Alimentari, Ambientali e Forestali (DAGRI), Università degli Studi di Firenze, Piazzale delle Cascine, 18, 50144 Firenze, Italy.*

[2] *Renewable Energy COnsortium for R&D (RE-CORD), Università degli Sudi di Firenze, Viale Morgagni 40, 50134 Firenze, Italy.*

Key words: agro-industrial residues, *Amaranthus cruentus*, bio methane potential, *Jatropha curcas* L., milk whey, pelargonium graveolens.

(*) Corresponding author:
enrico.palchetti@unifi.it

Citation:
PALCHETTI E., CALAMAI A., VERDI L., MASONI A., MARINI L., CHIARAMONTI D., 2019 - *Preliminary screening of agricultural feedstocks for anaerobic digestion*. - Adv. Hort. Sci., 33(3): 333-344

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 17 January 2019
Accepted for publication 12 April 2019

Abstract: The aim of this study is to evaluate the performances in the early stages of biogas production of various unconventional and low inputs crops, such as: kenaf (*Hibiscus cannabinus* L.), amaranthus (*Amarathus cruentus* L.), sorghum (*Sorghum bicolor* L.), and sunflower (*Helianthus annuus* L.). Moreover, according to a circular economy approach, that foreseen the re-use of all the materials, a wide range of agro-industrial residues were tested such as: pomace, olive oil cake, cow milk whey, ewe milk whey, beer residues, jatropha (*Jatropha curcas* L.) oil cake and pelargonium (*Pelargonium graveolens* L.) residues after essential oil extraction. The biogas production was estimated starting from the chemical composition of the substrates as well as through tests in bench's static reactors. The results showed that the use of silage from crops with reduced agronomic requests (kenaf and amaranthus) versus a conventional crop (corn) led to comparable, or even better, biogas production performances during the initial stages. Moreover, the performance of some residues from the milk industry allowed to conclude that the ewe milk whey can be considered a booster feedstock for the first phase of digestion. All the tested substrates produced a digestate suitable, according to the Italian rules, for soil fertilization or amendment.

1. Introduction

Biogas production from anaerobic digestion (AD) for electricity and heat generation represents a significant and well-established opportunity for farmers in the EU countries thanks to several reasons, such as: large technology availability and versatility, very attractive integration of the system in the agronomic practices and rotations, and the availability of incentives provided by the EU-MS to renewable energy generation. For all these reasons the biogas sector in Europe showed a remarkable increase during the last decade, with a global amount of energy produced in 2013 in the EU of 561 x 10^{-9} GJ (EurObserv'ER, 2014).

In this European context, the Italian biogas growth was mostly related

to the agriculture sector and almost 800 power plants based on agricultural biogas were operating at the end of 2012 with a total capacity of 650 MW (Patrizio *et al.,* 2015). The plants are concentrated in the northern part of the country, mainly fed with silage corn and manure, thanks to the livestock sector present there.

However the use of corn as feedstock, although it represents one of the most performing biomass in AD (with an average biogas production of 498 $m^3\ t^{-1}$ and a content of 53% of methane), exposes the biomass chain to the criticism of food-feed conflict. The largest part of the Italian biogas plants are fed with livestock manure and energy crops (62.2%), 17.7% of plants use only livestock manure and 20.1% use only energy crops and cereals (Carrosio, 2013). Nevertheless, the main constituent of the feedstock recipes is still represented by the dedicated crops (Dresseler *et al.*, 2012; Bacenetti *et al.*, 2013).

For all these reasons, the present work aimed at preliminary testing several unconventional biomass for the production of biogas in comparison with a traditional feedstock such as silage corn. These feedstocks belong to two main groups: dedicated crops, such as corn silage (as control), sorghum silage, kenaf silage, sunflower silage and amaranthus silage; and agro-industrial residues such as pelargonium residues, olive oil cake, jatropha oil cake, pomace, beer thrasher, cow milk whey and ewe milk whey.

As regards the agro-industrial residues, some of these have been selected since they are widely available in Europe. In fact, they are originated from processes such as olive oil extraction, wine beer and cheese factory, activities very common in this area.

Concerning the jatropha cake (from *Jatropha curcas* oil extraction process) and the residues of the essential oil extraction from *Pelargonium graveolens*, these two biomasses, requiring tropical climates, well fit to tropic areas, where short energy chain is often necessary due to the lack of grid connection and where biogas production could represent an interesting energetic chance.

The bio-methane potential (BMP) of the selected organic matter can be estimated through chemical analysis followed by the use of algorithms, to approximate the biogas and methane potential yield (Buswell and Symons, 1993), or assessed by a simulating small scale anaerobic digestion in laboratory, either batch wise or continuous (Angelidaki *et al.*, 2009; Kowalczyk *et al.*, 2013; Edward *et al.*, 2015).

The bio-methane potential of a biomass can be estimated from its elemental composition (carbon, oxygen, hydrogen, nitrogen and sulfur content) using the well-known Buswell's formula (1):

$$C_nH_aO_bN_cS_d + \left(n - \frac{a}{4} - \frac{b}{2} + \frac{3c}{4} + \frac{d}{2}\right) \bullet H_2O \rightarrow$$

$$\left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4} + \frac{3c}{8} + \frac{d}{4}\right) \bullet CO_2 + \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8} - \frac{d}{4}\right) \bullet CH_4 + c \bullet 0NH_3 + d \bullet H_2S$$

where represent the molar number of each element.

Results are calculated under standard conditions, according to Eq. (2):

$$CH_4 = \frac{\left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8} - \frac{d}{4}\right) \bullet 22.4}{(12n + a + 16b + 14c + 32d)}$$

where represent the molar number of each element and 22.4 is the coefficient that express the volume of 1 mole of perfect gas at standard conditions (0°C and 1 atm).

But several factors influence the estimation of Buswell's formula, such as the particles size, the retention time, the temperature, any unbalance in the biomass recipe, the recalcitrant molecules that might occur in the biomass, etc. and, in order to overcome these difficulties, a wide range of tests has been carried out during the last decades to detect the biomass bio-methane potential production. Bio-Methane potential (BMP) tests are very useful for this scope, as they provide a measure of the maximum amount of biogas or bio-methane produced per gram of volatile solids (VS) contained in the organic feedstock (Esposito *et al.,* 2012; Thomsena *et al.,* 2014).

The BMP methodology requires to control the substrate chemical composition, the operating temperature, the inoculum, the length of the trial and the output characterization (biogas and digestate composition).

Owens and Chynoweth (1993) and Angelidaki and Sanders (2004) proposed a BMP method based on a batch process, with a good stability due to the equilibrium between the symbiotic growth of the principal metabolic groups of bacteria; the methodology chosen for the present study refers to Angelidaki *et al.* (2009).

The use of dedicated crops for the biogas process fuelling is well known and a large amount of data is available in scientific literature since the '50s (Reinhold and Noak, 1956), showing a wide range of methane yield such as: VS 200-450 $m^3\ t^{-1}$ CH_4 corn, VS 384-426 $m^3\ t^{-1}$ CH_4 wheat (Schievano *et al.*, 2009; Wu *et al.*, 2010), VS 236-428 $m^3 t^{-1}$ CH_4 sorghum

(Windpassinger *et al.*, 2015), VS 242-324 m^3 t^{-1} CH_4 straw, VS 298-467 m^3 t^{-1} CH_4 grass (Zauner and Küntzel, 1986; Weiland, 2010; Wu *et al.*, 2010), VS 177-400 m^3 t^{-1} CH_4 sunflower (Schittenhelm, 2010; Weiland, 2010) and VS 355-409 m^3 t^{-1} CH_4 hemp (Heiermann *et al.*, 2009; Nges *et al.*, 2012) however, new crops are emerging in recent years (Molinuevo-Salces *et al.*, 2013; Mast *et al.*, 2014; Corno *et al.*, 2015).

Examples of crops not yet widely exploited for biogas production are kenaf (*Hibiscus cannabinus*) and amaranthus (*Amaranthus cruentus*). The first one represents a major feedstock for cellulose pulp (Nishinoa *et al.*, 2003) or for green building component production (Deka *et al.*, 2013) while amaranthus well fit to temperate environments, where short days varieties of tropical origin can enhance the biomass growth rather than the seeds production.

There is a common pre-treatment step among different biomass crops: the after harvest silage process. This is essential to simulate the usual practice in actual biogas plants because the process allows the biomass storage for several months.

As regards the by-products considered in the present article, no literature is available for geranium (*Pelargonium graveolens*), where leaves remaining after essential oil extraction can be used for AD. Similarly, jatropha (*Jatropha curcas*) cake, after oil extraction, could represent an interesting feedstock in some tropical areas.

No specific EU regulation or rules are actually available for the use of digestate as fertilizer or amendment, although its potential utilization can also reduce dependence on energy intensive mineral fertilizers, to further mitigate GHG emissions (Pöschl *et al.*, 2010). In Italy the only limitation is expressed by the Decreto del Ministero dell'Agricoltura 7.4.2006 (MPAF, 2006) stating that if the feedstock is animal manure also the digestate could be considered appropriate as fertilizer, otherwise its utilization is uncertain. Recently a new Italian rule (Legge, 7.10.2012, n. 134) declared that if the digestate is not originated from waste, it can be classified as a by-product usable in agriculture as soil amendment, but every Region applies this rule differently. The use of digestate as fertilizer or soil amendment is validated from several scientific articles (Haraldsen *et al.*, 2011; Mantovi *et al.*, 2011; Chen *et al.*, 2012; García-Sanchez *et al.*, 2015) but only a few works in literature are available regarding the digestate from these still unexploited (in most of the cases) sources.

2. Materials and Methods

Feedstock description

An experimental trial was conducted using twelve different substrates, some of which were dedicated crop specifically cultivated for this use and silaged after harvest (biomass), while the others residues came from various agricultural activities (agro-industrial by-products). The following Table 1 summarizes all the crop type and the origin of the different feedstock tested in the experiment.

The crops substrates were corn (*Zea mays* L.), sorghum (*Sorghum bicolor* L.), amaranthus (*Amaranthus cruentus* L.), kenaf (*Hibiscus cannabinus* L.) and sunflower (*Heliantus annuus* L.) cropped in experimental fields in the farm owned by the Istituto Tecnico Agrario (Florence, Italy) and transformed with the silage technique. A field experiment was carried out in 2012 during April - September, in the farm of the University of Florence, Italy. The seeds for the experimental field were collected from different sources (Table 2).

The research fields were located at latitude 43°47'07'' N and longitude 11°13'12'' E, with an altitude of about 40 m above mean sea level. Maximum and minimum temperature were respectively 29.8 and 17°C, with a mean of 24.6°C as recorded by Oregon Scientific WMR300. The soil texture was sandy-loam (67.6% and 20.7% respectively) with pH of 6.5. No fertilization was planned because the pre-

Table 1 - Feedstock description

No.	Feedstock name	Type	Origin
1	Corn	Green Silage	Italy
2	Sorghum	Green Silage	Italy
3	Pelargonium	Residues from distillation of essential oil, dry	Madagascar
4	Kenaf	Green Silage	Italy
5	Sunflower	Green Silage	Italy
6	Amaranth	Green Silage	Italy
7	Olive cake	Residues from mechanical oil extraction	Italy
8	Jatropha cake	Residues from mechanical oil extraction	Madagascar
9	Wine residues	Residues after fermentation	Italy
10	Cow milk whey	Residues after cheese production	Italy
11	Ewe milk whey	Residues after cheese production	Italy
12	Beer residues	Residues after fermentation	Italy

vious crop insisting on that area was a legume (*Phaseolus vulgaris* L.) and the nitrogen soil content was 1.4%.

Table 2 - Dedicated crops description

Species	Variety name	Seed provenance
Zea mays L.	Cisko	Syngenta Ltd
Sorghum bicolor L. (Moench)	Bulldozer	KWS Ltd
Hibiscus cannabinus L.	HIB 35	IPK seed bank
Helianthus annuus L.	PR64H41	Pioneer Ltd
Amaranthus cruentus L.	Perucho	DISPAA seed bank

The experimental scheme adopted was a completely randomized block with 3 repetition and each plot measured 4 x 5 m and was irrigated immediately after seeding, then irrigation plan was not necessary and the rainfall recorded during the cultivation months was 125.47 mm. Weed control was done manually in the early stages of plants growing.

Considering the restricted number of plants, harvest and ensilage were realized manually on 6 September 2012, collected biomass of each crop was chopped by Stayer Trito 1800 shredder and vigorously compacted into nylon bags. All the bags, hardly sealed, were left for two months in a Temperature controlled environment (25°C), to allow the fermentation processes and the conversion of chopped and pressed biomass in silage. After that, samples were collected in vacuum envelopes, by Valko Favola 310 vacuum packaging machine, with a total amount of 500 g for each crop.

The agro-industrial by-products substrates consisted in pomace, olive oil cake, cow and ewe milk whey and beer residues that were obtained directly from agro-industrial districts in the Tuscany Region, Italy (farm, winery, oil mill, dairy).

As regards the by products used as feedstock for AD: the ewe whey was collected from "Società Agricola Bacciotti Giovanna" an organic farm located in Mugello valley into the municipality of Scarperia - San Piero (Florence) that produces both the sheep milk and the diary.

The ewe whey is the residue of Ricotta cheese (ricotta) production, a typical Italian unripe cheese variety obtained through heat-induced (85-90°C) coagulation of whey proteins, after addition of acidifying agents. During this phase, coagulated whey proteins are divided by the liquid part that, in this case of study, represents the feedstock used for the biogas production and was collected in 1 dm^3 sterile plastic container.

The experimental samples of cow milk whey were sampled from "TRE P" diary located in Mugello Valley in the municipality of Scarperia - San Piero (Florence) and the milk used was produced by the "Emilio Sereni" farm located in Mugello Valley as well, where Frisian and Pardo-Alpina races are grown by organic livestock.

The cow milk whey is the liquid residue that separates from the solid mass obtained from the coagulation of casein, during the production of Mozzarella cheese. Mozzarella cooking is accomplished by melting the curd in hot water (60-85°C) and then working the molten curd by manually stretching and kneading, until the desired texture is achieved. During this step, 1 dm^3 of the residual liquid part was collected in sterile plastic container.

The olive cake came from the mill "Il Mandorlo" located in Trespiano (Florence) where the farm has olives trees grown by organic agriculture. This feedstock represents the 'cold extraction' process residue and it is obtained during extra-virgin olive oil production at a temperature below 27°C from mechanical pressing of the olive paste, using a traditional extraction system with hydraulic presses. It is constituted from a mixture of olive endocarp, olive pulp and skin, as well as pomace olive oil plus the water added in the olive mills. The olive varieties used for oil production were Frantoio, Moraiolo, and Leccino. During extra-virgin oil production, 1 kg of olive cake was collected and vacuum-sealed.

Wine residues came from the "Ornina" winery located in Castel Focognano, Arezzo. The winery produces red wine starting from the organic grapes grown in his own vineyards and the varieties cultivated are Sangiovese and Malvasia Nera. Winemaking process is carried out in stainless steel tanks during the fermentation step, then, the following maceration of grapes continues for about 10 days. The by-product of red vinification that was used as feedstock for biogas production, is the "fermented grape marc" composed of skins and seeds: in October 2012, 1 kg of this material was collected and vacuum-sealed.

Beer residues came from "Birra dell'Elba" brewery, located in Elba Island in the municipality of Portoferraio. The feedstocks used for the biogas trials is represented by the residues of the mashing step realized to produce unfiltered and not pasteurized beers, with low fermentation temperatures (8-12°C). In October 2012, 1 kg of these residues were collected and vacuum-sealed.

Pelargonium (*Pelargonium graveolens*) residues after the essential oil extraction and jatropha (*Jatropha curcas* L.) cake were also investigated. Both

these crops were produced in the same area of cultivation: South Madagascar, Ihorombé Region, village of Satrokala (22°19'39 S, 45°42'54'' E; altitude 1025 m above sea level), where the research farm is located with a characteristic rainfall of 1200 mm year^{-1} mostly concentrated between October until March.

The samples were collected during the agronomic season of 2012, during that period the mean temperatures followed the rainfall with the higher value during the winter season (max 26.2°C, Min 17.2°C) and the lower value during the summer (Max 19.7°C, Min. 10.1°C).

The soil texture is sandy-clay (50.6% and 38.9% respectively) with an average pH value of 5.5 and a general limited mass fraction of dry matter of Nitrogen (0.12%) and Carbon (1.97%).

Pelargonium (*Pelargonium graveolens*) was cropped during the agronomic season 2012 - 2013, from December to April in an open-field plantation in double line with a planting density 50.000 plants ha^{-1}. The cuttings, coming from the farm nursery, were irrigated immediately after transplanting for the proper establishment of the crop in the field and after that the water supply was guaranteed with a dripping irrigation system that supply 4 liters day^{-1} of water for each plant. Only organic manures were applied before the crop installation using cow manure.

After 4 months of growing, at the so called balsamic time, the crop was harvested and the essential oil extraction from the biomass was carried out by steam distillation in a 2 tons distillatory plant for 6 hour. The residues coming from this process, roughly 99% of the original biomass (the oil content is approximately 2 g kg^{-1}) is represented by moist leaves and stocks that have been collected and sun-dried for 48 hours before shipping to Italy.

Concerning the jatropha cake, it represents the residual part of the oil extraction process from seeds. The seeds were harvested from a 3 years old plantation of *Jatropha curcas* L. located in the same experimental fields previously described for pelargonium in Madagascar. The plantation was established starting from cuttings grown in the farm nursery for 6 months and transplanted in open field during the agronomic season 2009/2010, no irrigation was performed and the amount of 200 g of NPK fertilizer (10:10:10) was applied for each plant. The unique agronomical practice performed during the cultivation is the second year pruning at 1 m high. The seed harvest was performed during the dry season of 2012 and the oil extraction was carried out following the mechanical extraction method using a bench Komet screw press (Model CA59G) with a pressing capacity of 3-5 Kg seeds per hour and an electric Power of Drive Motor of 1.1 kW.

Both jatropha and pelargonium residues were divided in samples of 100 g and the total amount of 500 g for each crop was prepared for the shipping to Italy. Each sample was collected in a vacuum envelope, by Valko Favola 310 vacuum packaging machine, to avoid alterations during the transport to the Florence laboratory; in addition, the pelargonium residues for AD have been rehydrated adding water 3 days before the beginning of the trial.

Physical-chemical and energetic analysis

Different laboratory equipment and methodologies were utilized for the analysis of substrates that were prepared through a cutting mill (mod. SM 300, Retsch). Moisture (UNI 14774-1:2009) and ash content (UNI 14775: 2010) were measured using a LECO 701 TGA. CHNOS content was measured using a Truespec CHN and S (LECO) (UNI CEN/TS 15104; KTBL, 2015). Data from these analyses were utilized to calculate the theoretical production of biogas for each substrate according to Buswell and Symons (1993).

The digested sludge was analyzed using the same technologies described above in order to estimate its potential use in agriculture as a soil improver. Furthermore the calorific value was measured by Isoperibolic calorimeter AC 500, Leco.

The total solid (TS), the volatile solid (VS) and the ash content of substrates were determined through a STF N-80, Falc stove. Each sample was weighted and placed in stove for 105°C until a constant weight was reached, after that, the dried material was burnt in a muffle furnace at 550°C and used for the determination of the raw ash content. The organic dry matter was calculated by subtracting the raw ash content from the dry matter.

Specific biogas and methane yield

Static reactor description. The static reactor was a 100 cm^3 glass vessel hermetically closed and placed on a heating plate connected to a thermocouple to control the constant heating (about 45°C) with a continuous monitoring of the system temperature. 4 repetitions were simultaneously carried out for each substrate. The measurement and temporary storage for the produced biogas consisted in a graduate syringe (30 cm^3) placed above the cap with the needle inserted into the rubber membrane and sealed with several layers of parafilm.

The ratio between volatile solids (VS) in the substrate and VS in the inoculum was 0.5. The produced amount of biogas was monitored on a daily basis for 10 days and then until there was no more biogas production.

Regular weekly analysis of inoculum and pH level in the reactors were carried out to evaluate the activity of inoculum and the progress level.

The qualitative analysis of biogas production was carried out through samples performed with a glass microsyringe and successive injection in GCMS (Shimadsu) for the gas chromatography determination. The biogas composition was measured with mass spectrometer gas chromatography GC-MS (GC - 2010 and QP 2010, Shimadsu).

Statistical analysis

The data generated in the present work belong to three major groups: i) theoretical biogas production with Buswell's formula; ii) biogas production of the different substrates from static digestion; iii) substrate and digestate chemical characterization.

Each different data was submitted to a specific statistical analysis and therefore: as regards type 1, the accuracy of Buswell's formula was evaluated through the calculation of standard deviation of the delta between the produced and measured biogas, for each substrate. The standard deviation comparison between the substrates was then evaluated with the "chi square" method.

ANOVA analysis on type 2 data was performed on the biogas production on three key moments: BioGas t_1 - the biogas production expressed in mL g^{-1} VS measured at the end of first day of anaerobic digestion; BioGas t_f - the cumulate final biogas production expressed in mL g^{-1} VS measured at day number 9; $IBRt_{(2\text{-}8)}$ - Increasing Biogas Rate (IBR) production calculated with the following formula (3):

$$IBR = \sum_{2}^{8}\left[\frac{Biogt_{tn}}{Biogt_{tn-1}}\right]$$

where $Biogt_{tn}$ represents the biogas production per day.

The fixed model of analysis of variance was applied using the statistical software SPSS IBM, and the significance of the variance was tested with the Tukey's test.

Regarding the biogas production trend during the digestion process, data were evaluated through the analysis of the regression within each different substrates. In addition, a regression analysis of the whole data set was carried out. The linear and the polynomial regression approximation were calculated and their significance tested according to ANOVA analysis and Fisher test. With respect to type 3 data, a simple correlation between the substrate and digestate composition was performed.

3. Results and Discussion

Accuracy of Buswell estimation of innovative substrates for AD production

Based on the chemical composition of the different substrates showed in Table 3, the potential biogas yields have been calculated using the Buswell's formula.

The Buswell estimation was applied to all the feedstock under investigation (Table 4), however, it provided acceptable values compared to bibliography (ASTM, 1998) only for some of these, specifically corn (Oslaj *et al.*, 2019), sorghum (Wannasek *et al.*, 2017), pelargonium (Gamal-El-Din *et al.*, 2012, Marsili Libelli

Table 3 - Feedstock elemental composition

No.	Feedstock	Moisture (%)	Ash (db) (%)	C (db) (%)	H (db) (%)	N (db) (%)	S (db) (%)	O (db) (%)	C/N ratio	VS ratio	TS (%)
1	Corn	81.00	4.31	50.67	6.20	0.83	0.20	37.79	61.05	71.42	19.00
2	Sorghum	80.00	7.65	46.46	5.51	0.96	0.17	39.25	48.40	84.61	20.00
3	Pelargonium	19.00	10.67	46.46	5.19	1.04	0.20	36.44	44.67	94.00	81.00
4	Kenaf	76.01	7.93	57.40	4.06	1.10	0.13	29.38	52.18	97.70	23.99
5	Sunflower	78.70	8.50	62.95	3.14	1.10	0.11	24.20	57.23	90.00	21.33
6	Amaranth	74.00	5.45	41.40	4.66	1.70	0.20	46.68	24.35	77.80	26.00
7	Olive cake	64.60	4.40	63.90	6.88	0.80	0.20	23.82	79.88	95.60	35.40
8	Jatropha cake	52.40	1.96	58.55	6.08	3.40	0.20	29.81	17.22	98.00	47.62
9	Wine residues	69.02	1.14	32.05	8.13	2.20	0.20	56.29	14.57	96.50	30.98
10	Cow milk whey	99.10	0.06	1.13	11.00	0.30	0.00	87.51	3.77	100.00	0.91
11	Ewe milk whey	92.50	0.79	3.41	10.70	0.48	0.00	84.62	7.10	93.30	7.56
12	Beer residues	72.30	0.91	44.00	6.43	1.80	0.05	46.82	24.44	95.60	27.70

Table 4 - Buswell's formula estimation, biogas experimental production and comparison with Corn's biogas yield

No.	Feedstock	Biogas production estimation by Buswell's formula ($m^3\ t^{-1}$)	Trial measurement biogas at day 9 ($m^3\ t^{-1}$)	Biogas yield at day 9 in comparison to Corn (%)
1	Corn	990.51	359.50	-
2	Sorghum	940.83	322.75	- 10.22%
3	Pelargonium	973.02	217.25	- 39.57%
4	Kenaf	1165.39	370.50	+ 3.06%
5	Sunflower	1285.77	150.68	- 58.09%
6	Amaranth	818.29	156.14	- 56.57%
7	Olive cake	1250.31	310.67	- 13.58%
8	Jatropha cake	1117.00	222.25	- 38.18%
9	Wine residues	829.3	66.85	- 81.40%
10	Cow milk whey	21.10	245.93	- 31.59%
11	Ewe milk whey	64.16	483.87	+ 34.60%
12	Beer residues	829.3	248.43	- 30.90%

et al., 2014) and beer residues (Maier, 2015; Mugodo *et al.*, 2017), with a biogas estimated productions equal to 990.51 $m^3\ t^{-1}$, 940.83 $m^3\ t^{-1}$, 973.02 $m^3\ t^{-1}$ and 829.30 $m^3\ t^{-1}$ respectively. For the other silage feedstock, kenaf (Saba *et al.*, 2015), sunflower (Dubrovskis *et al.*, 2012; Adamovics and Dubrovski, 2015; Markou *et al.*, 2017) and amaranth (Sitkey *et al.*, 2013; Minzanova *et al.*, 2018), the Buswell's formula application provides unrealistic values of 1165.39 $m^3\ t^{-1}$, 1285.77 $m^3\ t^{-1}$ and 818.29 $m^3\ t^{-1}$ respectively. Similar high values of 1250.31 $m^3\ t^{-1}$ and 1117.00 $m^3\ t^{-1}$ arise also respectively from the Buswell estimation performed among the olive oil cake (Tekin and Dalgic, 2000; Battista *et al.*, 2015; Valenti *et al.*, 2017) and jatropha oil cake (Staubmann *et al.*, 1997; Grimsby *et al.*, 2013; Jingura and Kamusoko, 2018) while the cow milk whey and ewe milk whey (Battista *et al.*, 2015) record limited and unacceptable low value of 21.10 $m^3\ t^{-1}$ and 64.16 $m^3\ t^{-1}$ respectively. An acceptable (Failin and Restuccia, 2014; Mugodo *et al.*, 2017) level of accuracy could lie on the Buswell estimation performed over wine residues with: 829.30 $m^3\ t^{-1}$.

Therefore the reliability of the biogas production methodology among the substrates was evaluated in comparison with the corn silage biogas production observed during the experiment (Table 4).

A general lower production, when compared to corn silage biogas yield, can be observed among the majority of the feedstock and this reduced production ranges from admissible values of 10.22% and 13.58% of sorghum and olive oil cake respectively, until 81.40% of wine residues.

On the contrary, a positive value of 34.60% is observed from the comparison between corn and ewe milk whey, probably due to the booster effect of this special milk whey. This difference is even wider when the ewe milk whey biogas production is compared to the theoretical biogas production of 16.30 $m^3\ t^{-1}$ expressed by Buswell's formula application.

Finally, as regard the kenaf silage, it showed a limited increase of 3.06% in biogas production at day 9, when compared to the corn biogas production.

Biogas production from static digestion of the different substrates

The ANOVA analysis performed on biogas production data showed a significant effect ($P<0.001$) of the substrate.

As regards the $BioGas_{(t1)}$ (Fig. 1), the substrates that show a significantly higher production at the first day of digestion are sorghum silage, pelargonium residues and beer residues, with a production of 36.75; 35.25 and 35.23 mL g^{-1} VS biogas respectively. On the opposite, a very limited performance in terms of biogas production was observed for cow milk whey, wine residues, amaranth silage and jatropha oil cake with a production of: 1.73, 9.37, 10.00 and 13.37 mL g^{-1} VS of biogas respectively.

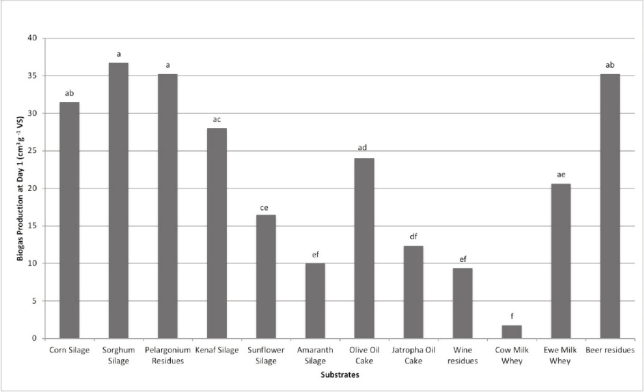

Fig. 1 - $BioGas_{(t1)}$ experimental production ($cm^3\ g^{-1}$ VS) for the different substrates. Different letters means significative differences between substrates according to Bonferroni Multiple Comparisons Test ($P<0.05$).

Among all the remaining substrates, it is possible to identify substrates that produce a large amount of biogas compared to the others such as the corn silage with 31.50 mL g^{-1} VS of biogas. Finally, kenaf silage, olive oil cake, ewe milk whey and sunflower silage generated a reduced quantity of biogas but significantly higher than cow milk whey.

The cumulate final biogas production BioGas $t_{(f)}$ at the end of day 9 (Fig. 2) shows instead a very different behavior. The most productive substrate is the ewe milk whey, with a production of 488.87 mL g^{-1} VS of biogas, while the smaller amounts of biogas were produced from kenaf silage and corn silage, 370.50 and 359.50 mL g^{-1} VS biogas respectively.

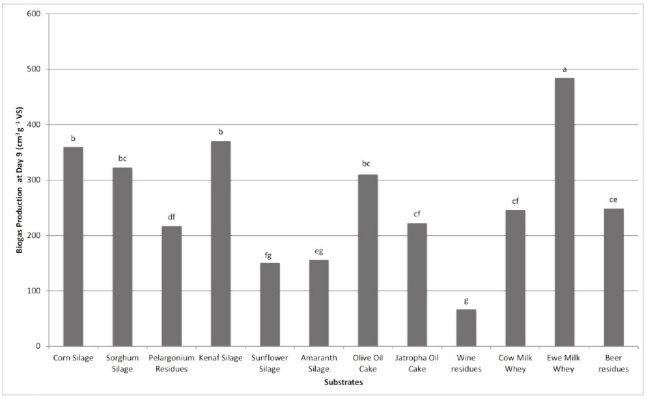

Fig. 2 - BioGas$_{(t9)}$ cumulate production (cm^3 g^{-1} VS) the end of day 9 for different substrates. Different letters means significative differences between substrates according to Bonferroni Multiple Comparisons Test (P<0.05).

At the opposite, the wine residues were the worst performing substrates with only 66.87 mL g^{-1} VS biogas, significantly differing from the majority of the substrates tested during the experimental campaign; only amaranth silage and sunflower silage had a statistically similar behavior, with 156.14 and 150.68 mL g^{-1} VS biogas production respectively. All the other substrates tested show intermediate levels of production: only sorghum silage and olive oil cake were similar to corn silage and kenaf silage.

The IBRt$_{(2-8)}$ ANOVA analysis allowed us to identify the cow milk whey (Fig. 3) as the substrate having the fastest growing rate of biogas production during the intermediate period of anaerobic digestion (IBR=1.59). A good IBR performance was observed also by ewe milk whey (IBR= 1.48) and jatropha oil cake (IBR=1.41) while the pelargonium residues, with an average value of 1.25, achieve lower IBR value similar to beer and wine residues.

Observing the overall tendency of cumulate biogas production (Fig. 4) with the support of the regression analysis that showed significance for the overall

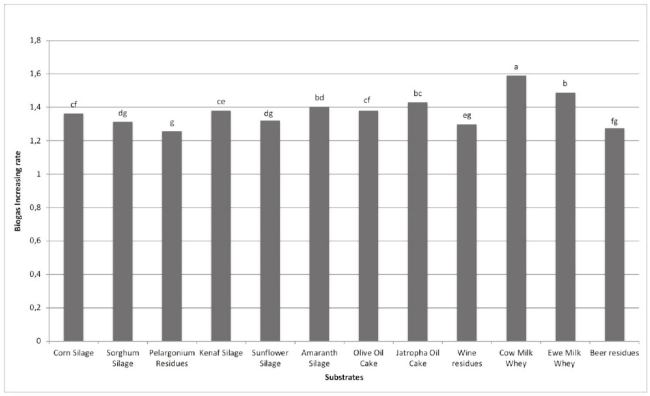

Fig. 3 - IBR$_{t(2-8)}$ values for different substrates. Different letters means significative differences between substrates according to Bonferroni Multiple Comparisons Test (P<0.05).

variance of the regression and, when performed within each substrate, for the linear and polynomial regression as shown in Table 5, it is possible to make some general assumptions.

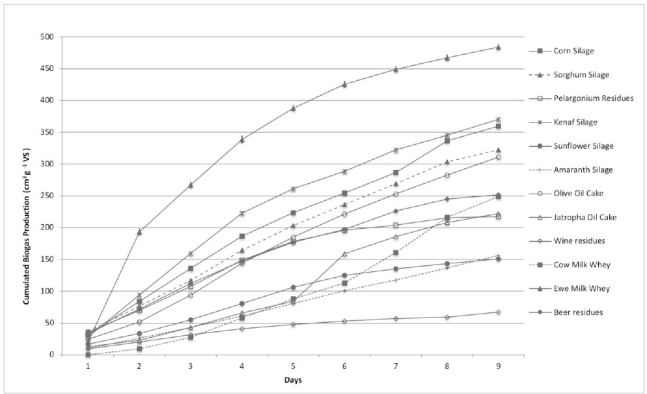

Fig. 4 - Overall tendency of biogas production among the different substrates.

Ewe milk whey substrate showed a cumulate biogas production superior during the entire digestion period, with the exclusion of the first day. The tendency is therefore a fast growth of biogas production during the whole experimental campaign (Table 5, Figs. 4 and 5).

At the opposite, residues showed a limited increase of biogas production during the entire cycle, leading to the lower biogas (Fig. 4).

Kenaf silage, corn silage, sorghum silage and olive oil cake have a similar behavior during the digestion process, with similar final and intermediate biogas yields (Fig. 4).

More complex trends were observed for the remaining substrates; in effect, it is possible to identify three main behaviors:

Beer residues and pelargonium residues showed a

Table 5 - Linear and polynomial regressions equations. R2 values and significance for the different substrate tested

N.	Substrate	Linear regression		Polynomial regression	
		Equation	R^2 (Sign)	Equation	R^2 (Sign)
1	Corn silage	$Y = 40.629\ x + 7.770$	0.976 **	$Y = -1.57\ x^2 + 56.35\ x - 21.03$	0.983 NS
2	Sorghum silage	$Y = 36.671\ x + 8.868$	0.919 **	$Y = -1.33\ x^2 + 50.03\ x - 15.63$	0.925 NS
3	Pelargonium residues	$Y = 23.400\ x + 35.389$	0.864 NS	$Y = -3.07\ x^2 + 54.17\ x - 21.05$	0.940 *
4	Kenaf silage	$Y = 41.904\ x + 22.951$	0.954 NS	$Y = -3.82\ x^2 + 80.18\ x - 47.25$	0.995 **
5	Sunflower silage	$Y = 17.584\ x + 4.861$	0.947 NS	$Y = -1.33\ x^2 + 31.15\ x - 15.52$	0.974 *
6	Amaranth silage	$Y = 18.43\ x + 10.837$	0.702 **	$Y = 0.14\ x^2 + 16.94\ x - 8.11$	0.702 NS
7	Olive oil silage	$Y = 37.249\ x + 12.135$	0.980 **	$Y = -1.05\ x^2 + 47.79\ x - 31.48$	0.984 NS
8	Jatropha oil silage	$Y = 29.567\ x + 36.483$	0.867 **	$Y = 0.91\ x^2 + 20.37\ x - 19.62$	0.871 NS
9	Wine residues	$Y = 6.833\ x + 8.722$	0.317 **	$Y = 0.61\ x^2 + 12.99\ x - 2.56$	0.330 NS
10	Cow milk whey	$Y = 32.300\ x + 59.110$	0.962 NS	$Y = -2.58\ x^2 + 6.49\ x - 11.79$	0.990 **
11	Ewe milk whey	$Y = 52.108\ x + 76.440$	0.843 NS	$Y = -8.36\ x^2 + 135.72\ x - 76.83$	0.954 **
12	Beer residues	$Y = 27.833\ x + 23.194$	0.956 NS	$Y = -1.57\ x^2 + 56.35\ x - 21.03$	0.983 *

** significative for P<0.01; * significative for P<0.05; NS= not significative.

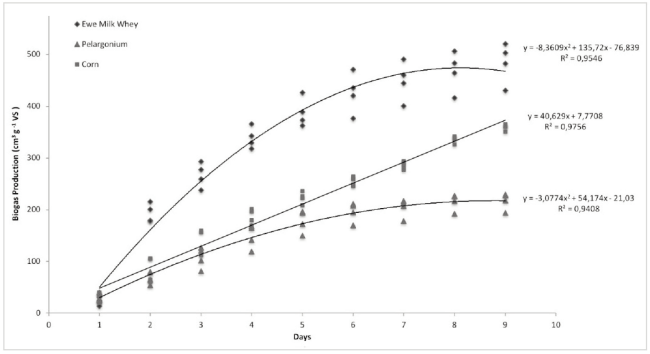

Fig. 5 - Regression (linear and polynomial) of ewe milk whey, corn silage and pelargonium residues.

common trend until day 5, similar to the other groups, and then diverge, with a reduction in the increasing rate of biogas production.

Jatropha oil residues and cow milk whey, that generally showed a low level of biogas production, had an initial poor production until day 5 followed by a certain increase during the second period.

Sunflower silage and amaranth silage showed a limited but continuous increase in biogas production from day 1 to 9.

Digestate chemical characterization

Digestate from the AD of different substrates was chemically characterized (Table 6).

The use of digestate as fertilizer or soil amendment is suggested when an appropriate N content (1-4%) is linked to a C/N ratio between 10 and 20 (Haraldsen *et al.*, 2011; Mantovi *et al.*, 2011; Chen *et al.*, 2012; Garcia-Sanchez *et al.*, 2015): for these reasons, all the different tested feedstocks used were reasonably suitable for this purpose. The high C/N ratio of some of them, such as jatropha cake (19.13) and amaranth (17.56), seems very interesting towards microbial processing soil bacteria.

4. Conclusions

The Buswell's formula effectiveness, estimating the biogas production from various feedstock, was investigated and results of the analysis showed that while it approximates sufficiently well the biogas production rate during the early estimation for conventional feedstock, it does not perform adequately

Table 6 - Digestate elemental composition

No.	Digestate	Moisture (%)	Ash (db) (%)	C (db) (%)	H (db) (%)	N (db) (%)	S (db) (%)	HHV (db) (MJ/Kg)	C/N ratio
1	Corn	95.55	0.89	39.23	5.48	3.11	0.33	16.45	12.61
2	Sorghum	95.40	0.92	39.25	5.54	2.85	0.31	16.57	13.77
3	Pelargonium	95.00	1.00	38.67	5.39	2.73	0.36	16.08	14.16
4	Kenaf	95.26	6.40	40.00	5.13	2.72	0.40	16.65	14.71
5	Sunflower	94.24	5.20	39.00	5.01	2.61	0.32	16.51	14.94
6	Amaranth	94.40	2.00	37.75	5.28	2.15	0.34	15.18	17.56
7	Olive cake	94.50	1.80	39.40	5.34	2.87	0.34	16.45	13.73
8	Jatropha cake	94.60	4.05	39.60	5.36	2.07	0.31	15.99	19.13
9	Wine residues	93.20	4.00	42.97	5.75	2.73	0.32	17.37	15.74
10	Cow milk whey	97.30	3.70	34.35	4.75	2.40	0.38	15.10	14.31
11	Ewe milk whey	96.90	3.75	36.07	5.03	2.73	0.38	14.87	13.21
12	Beer residues	93.50	4.10	41.80	5.55	2.72	0.30	16.87	15.37

when applied to un-conventional substrates, with a general over estimation for the crop silage or crop residue, and with an under estimation for the milk residues. Consequently, the application of the Buswell formula to test new substrates should be limited to a preliminary survey and coupled with a BMP test.

The tests carried out on these new substrates led to several conclusions.

The ewe milk whey represents a good booster product in AD and should thus be coupled in a limited percentage with conventional substrates, such as corn silage or similar.

The cow milk whey did not perform equally well as booster product, despite it shows the best increasing biogas rate (IBR).

Corn and sorghum silage showed a common behavior in AD, as expected.

Kenaf silage gave an interesting and remarkable performance, as this low input crop produces almost the same amount of biogas than corn and sorghum. Therefore, kenaf could represent a promising alternative to conventional corn silage.

The olive oil cake moreover, with its constant and increasing biogas production over the whole period, could be an alternative to kenaf silage but, due to an expected reduction during the following days after day 9, can be use only in limited percentage.

Sunflower and amaranth silage, despite a limited biogas production, might represents the most sustainable crops due to their limited requests of water, fertilizers and labor needs.

Regarding wine and beer residues they both did not perform sufficiently well in anaerobic digestion; its energetic use could be probably better take advantage in direct combustion or in charcoal production.

Specific considerations should be carried out for jatropha oil cake and pelargonium residues, since they both represent a biomass largely available in the tropical areas (Southern Madagascar) and despite the low biogas production, is possible to speculate the creation of a local self-sufficient production of biogas to power the extraction systems in Madagascar.

References

ADAMOVICS A., DUBROVSKI V., 2015 - *Anaerobic digestion of sunflowers and amaranth silages with catalyst metaferm,* pp. 385-389. - In: ZEVERTE-RIVZA S. (ed.) *Nordic view to sustainable rural development*. Proceeding of the 25th NJF Congress, June 16-18, Riga, Latvia, pp. 512.

ANGELIDAKI I., ALVES M.M., BOLZONELLA D., BORZACCONI L., CAMPOS J.L., GUWY A.J., KALYUZHNYI S., JENICEK P., VAN LIER J.B., 2009 - *Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays.* - Water Sci. Technol., 59: 927-934.

ANGELIDAKI I., SANDERS W., 2004 - *Assessment of the anaerobic biodegradability of macropollutants.* - Rev. Environ. Sci. Bio., 3(2): 117.

ASTM, 1998 - *ASTM D 4239-94: Standard test methods for sulfur in the analysis sample of coal and coke using high temperature tube furnace combustion methods* - Annu. Book ASTM Stand., 05.05: 397-400.

BACENETTI J., NEGRI M., FIALA M., GONZÁLEZ-GARCÍA S., 2013 - *Anaerobic digestion of different feedstocks: Impact on energetic and environmental balances of biogas process.* - Sci. Total Environ., 463-464: 541-551.

BATTISTA F., FINO D., ERRIQUENS F., MANCINI G., RUGGERI B., 2015 - *Scaled-up experimental biogas production from two agro-food waste mixtures having high inhibitory compound concentrations.* - Renew. Energ., 81: 71-77.

BUSWELL A.M., SYMONS G.E., 1993 - *The methane fermentation of carbohydrates.* - J. Am. Chem. Soc., 55: 2028-2039.

CARROSIO G., 2013 - *Energy production from biogas in the Italian countryside: Policies and organizational models*. - Energy Policy, 63: 3-9.

CEN/TS 15104, 2011 - *Solid biofuels - Determination of total content of carbon, hydrogen and nitrogen - Instrumental methods.* - CEN, Comité Europeen de Normalisation, Brussels, Belgium.

CHEN S., CHEN B., SONG D., 2012 - *Life-cycle energy production and emissions mitigation by comprehensive biogas–digestate utilization.* - Bioresour. Technol., 114: 357-364.

CORNO L., PILU R., TAMBONE F., SCAGLIA B., ADANI F., 2015 - *New energy crop giant cane (*Arundo donax *L.) can substitute traditional energy crops increasing biogas yield and reducing costs.* - Bioresour. Technol., 191: 197-204.

DEKA H., MISRAA M., MOHANTY A., 2013 - *Renewable resource based "all green composites" from kenaf biofiber and poly (furfuryl alcohol) bioresin.* - Ind. Crops Prod., 41: 94-101.

DRESSLER D., LOEWEN A., NELLES M., 2012 - *Life cycle assessment of the supply and use of bioenergy: impact of regional factors on biogas production.* - Int. J. Life Cycle Ass., 17(9): 1104-1115.

DUBROVSKIS V., KOTELENECS V., CELMS A., ZABAROVSKIS E., 2012 - *Biomass cofermentation for biogas producing,* pp. 560-565. - In: GALINS A., and A. LAIZANS (eds.) *Proceedings of 11th International Scientific Conference:* Engineering for Rural Development, May 24-25,

Jelgava, Latvia, pp. 680.

EDWARD M., EDWARDS S., EGWU U., SALLIS P., 2015 - *Biomethane potential test (BMP) using inert gas sampling bags with macroalgae feedstock*. - Biomass Bioenergy, 83: 516-524.

ESPOSITO G., FRUNZO L., LIOTTA F., PANICO A., PIROZZI F., 2012 - *Bio-methane potential tests to compare the biogas production from the digestion and co-digestion of complex organic substrates*. - Open Environ. Eng. J., 5: 1-8.

EUROBSERV'ER, 2014 - *Biogas Barometer*. - EurObserv'ER, November 2014: 1-12.

FAILIN S., RESTUCCIA A., 2014 - *Methane potentials from grape marc by a laboratory scale plant*. - Appl. Math. Sci., 8(132): 6665-6678.

GAMAL-EL-DIN H., EL-BASSEL A., EL-BADRY M., 2012 - *Biogas production from some organic wastes,* pp. 463-473. - In: EL-HALWAGI M.M. (ed.) *Biogas technology, transfer and diffusion*. Springer, Dordrecht, Netherlands, pp. 720.

GARCÍA-SANCHEZ M., SILES A.J., CAJTHAML T., GARCÍA-ROMERA I., TLUSTOŠ P., SZÁKOVÁ J., 2015 - *Effect of digestate and fly ash applications on soil functional properties and microbial communities*. - Eur. J. Soil Biol., 71: 1-12.

GRIMSBY L.K., FJØRTOFT K., BERNT A.J., 2013 - *Nitrogen mineralization and energy from anaerobic digestion of Jatropha press cake.* - Energy Sustain. Dev., 17: 35-39.

HARALDSEN T.K., ANDERSEN U., KROGSTAD T., SØRHEIM R., 2011 - *Liquid digestate from anaerobic treatment of source separated household waste as fertilizer for barley,* pp. 564-569. - In: LASARIDI K., T MANIOS, W. BIDLINGMAIER (eds.) *Proceedings of the 7th International ORBIT 2010 Conference*, June 29-July 3, Heraklion, Crete, Greece.

HEIERMANN M., PLÖCHL M., LINKE B., SCHELLE H., HERMANN C., 2009 - *Biogas crops. Part I: specifications and suitability of field crops for anaerobic digestion*. - Agricultural Engineering International: the CGIR Ejournal Manuscript 1087, 11: 1-11.

JINGURA R.M., KAMUSOKO R., 2018 - *Technical options for valorisation of jatropha press-cake: a review*. - Waste Biomass Valor., 9: 701-713.

KOWALCZYK A., HARNISCH E., SCHWEDE S., GERBER M., SPAN R., 2013 - *Different mixing modes for biogas plants using energy crops.* - Appl. Energy, 112: 466-472.

KTBL, 2015 - *The online european feedstock atlas*. - Kuratorium für Technik und Bauwesen in der Landwirtschaft, 94, Germany.

LEGGE 7 agosto 2012 n. 134 - *Conversione in legge, con modificazioni, del decreto-legge 22 giugno 2012, n. 83, recante Misure urgenti per la crescita del Paese, art. 52 "Disposizioni in materia di tracciabilità dei rifiuti"*. - Gazzetta Ufficiale, n. 187 dell'11 agosto 2012.

MAIER T., 2015 - *Digestion of brewery residues - potential and challenges* - Final Conference FABbiogas, Brussels, Belgium, pp. 1-15.

MANTOVI P., VERZELLESI F., BATTILANI A., 2011 - *Acqua e digestato bovino in manichetta*. *Speciale fertirrigazione con liquami*. - Agricoltura, 5: 75-77.

MARKOU G., BRULE M., BALAFOUTIS A., KORNAROS M., GEORGAKAKIS D., PAPADAKIS G., 2017 - *Biogas production from energy crops in northern Greece: economics of electricity generation associated with heat recovery in a greenhouse.* - Clean Technol. Environ. Policy, 19: 1147-1167.

MARSILI LIBELLI I., VERDI L., PALCHETTI E., CHIARAMONTI D., 2014 - *Physic-chemical characterization and biogas production potential evaluation of alternative agricultural substrates.* - Proceeding of the 22nd European Biomass Conference and Exhibition, 23-26 June, Hamburg, Germany.

MAST B., LEMMERB A., OECHSNERB H., REINHARDT-HANISCHB A., CLAUPEINA W., GRAEFF-HÖNNINGER S., 2014 - *Methane yield potential of novel perennial biogas crops influenced by harvest date*. - Ind. Crops Prod., 58: 194-203.

MINZANOVA S.T., MIRINOV V.F., BELOSTOTSKII D.E., MINDUBAEV A.Z., MIRONOVA L.G., GINS M.S., GINS V.K., KONONKOV P.F., MILYUKOV V.A., 2018 - *Materials derived from* Amaranthus cruentus *L. used as co-substrates can intensify methanogenesis during bioconversion of organic waste*. - Agricultural Biology, 53(1): 209-217.

MOLINUEVO-SALCES B., LARSEN S.U., AHRING B.K., UELLENDAHL H., 2013 - *Biogas production from catch crops: Evaluation of biomass yield and methane potential of catch crops in organic crop rotations*. - Biomass Bioenergy, 59: 285-292.

MPAF, 2006 - "*Criteri e norme tecniche generali per la disciplina regionale dell'utilizzazione agronomica degli effluenti di allevamento", di cui all'art. 38 del decreto legislativo 11 maggio 1999, n. 152*. - Gazzetta Ufficiale n. 109 del 12 maggio 2006, Supplemento Ordinario, n. 120.

MUGODO K., MAGAMA P.P., DHAVU K., 2017 - *Biogas production potential from agricultural and agro- processing waste in South Africa.* - Waste Biomass Valor., 8: 2383-2392.

NGES I.A., ESCOBAR F., FU X., BJORNSSON L., 2012 - *Benefits of supplementing an industrial waste anaerobic digester with energy crops for increased biogas production*. - Waste Manag., 32: 53-59.

NISHINOA T., HIRAOA K., KOTERAA M., NAKAMAEA K., INAGAKIB H., 2003 - *Kenaf reinforced biodegradable composite.* - Compos. Sci. Technol., 63: 1281-1286.

OSLAJ M., SUMENJAK T.K., LAKOTA M., VINDIS P., 2019 - *Parametric and nonparametric approaches for detecting the most important factors in biogas production.* - Pol. J. Environ. Stud., 28(1): 291-301.

OWENS J.M., CHYNOWETH D.P., 1993 - *Biochemical methane potential of municipal solid-waste (MSW) components*. - Water Sci. Technol., 27(2): 1-14.

PATRIZIO P., LEDUC S., CHINESE D., DOTZAUER E., KRAXN-

ER F., 2015 - *Biomethane as transport fuel. A comparison with other biogas utilization pathways in northern Italy*. - Appl. Energ., 157: 25-34.

PÖSCHL M., WARD S., OWENDE P., 2010 - *Evaluation of energy efficiency of various biogas production and utilization pathways*. - Appl. Energ., 87(11): 3305-3321.

REINHOLD F., NOAK W., 1956 - *Laboratoriumsversuche uber die Gasgewinnung aus landwirtsschaftlichen Stoffen*. - In: LIEBMANN H. (ed.) *Gewinnung und Verwertung von Methan aus Klärschlamm und Mist*. Verlag R. Oldenbourg, Munchen, Germany, pp. 343.

SABA N., JAWAID M., HAKEEM K.R., PARIDAH M.T., KHALINA A., ALOTHMAN O.Y., 2015 - *Potential of bioenergy production from industrial kenaf (*Hibiscus cannabinus *L.) based on Malaysian perspective.* - Renew. Sust. Energ. Rev., 42: 446-459.

SCHIEVANO A., D'IMPORZANO G., ADANI F., 2009 - *Substituting energy crops with organic wastes and agro-industrial residues for biogas production*. - J. Environ. Manage., 90: 2537-2541.

SCHITTENHELM S., 2010 - *Effect of drought stress on yield and quality of maize/sunflower and maize/sorghum intercrops for biogas production*. - J. Agron. Crop Sci., 196: 253-261.

SITKEY V., GADUS J, KLISKY L., DUDAK A., 2013 - *Biogas production from amaranth biomass*. - Acta Regionalia et Environmentalica, 10(2): 61-64.

STAUBMANN R., FOIDL N., GUBITZ G.M., LA ERTY R.M., ARBIZU V.M.V., STEINER W., 1997 - *Biogas production from* Jatropha curcas *press-cake*. - Appl. Biochem. Biotechnol., 63-65: 457-467.

TEKIN A.R., DALGIC A.C., 2000 - *Biogas production from olive pomace*. - Resour. Cons. Recycl., 30: 301-313.

THOMSENA S.T., SPLIIDB H., ØSTERGÅRDA H., 2014 - *Statistical prediction of biomethane potentials based on the composition of lignocellulosic biomass*. - Bioresour. Technol., 154: 80-86.

UNI EN 14774-1, 2009 - *Solid biofuels. Determination of moisture content - Oven dry method - Part 1: Total moisture - Reference method* - UNI, Ente Italiano di Normazione.

UNI EN 14775, 2010 - *Solid biofuels. Determining the ash content*. - UNI, Ente Italiano di Normazione.

VALENTI F., PORTO S.M.C., CASCONE G., ARCIDIACONO C., 2017 - *Potential biogas production from agricultural by-products in Sicily. A case study of citrus pulp and olive pomace.* - J. Agric. Eng., 48(4): 1-19.

WANNASEK L., ORTNER M., AMON B., AMON T., 2017 - *Sorghum, a sustainable feedstock for biogas production? Impact of climate, variety and harvesting time on maturity and biomass yield*. - Biomass Bioenergy, 106: 137-145.

WEILAND P., 2010 - *Biogas production: current state and perspectives*. - Appl. Microbiol. Biotechnol., 85: 849-860.

WINDPASSINGER S., FRIEDT W., FRAUEN M., SNOWDON R., WITTKOP B., 2015 - *Designing adapted sorghum silage types with an enhanced energy density for biogas generation in temperate Europe*. - Biomass Bioenergy, 81: 496-504.

WU X., WANYING Y., ZHU J., MILLER C., 2010 - *Biogas and CH_4 productivity by co-digesting swine manure with three crop residues as an external carbon source.* - Bioresour. Technol., 101: 4042-4047.

ZAUNER E., KÜNTZEL U., 1986 - *Methane production from ensiled plant material.* - Biomass, 10: 207-223.