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Opportunities for Green Energy through Emerging Crops: Biogas Valorization of *Cannabis sativa* L. Residues

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Abstract: The present work shows the experimental evidence carried out on a pilot scale and demonstrating the potential of *Cannabis sativa* L. by-products for biogas production through anaerobic digestion. While the current state-of-the-art tests on anaerobic digestion feasibility are carried out at the laboratory scale, the here described tests were carried out at a pilot-to-large scale. An experimental campaign was carried out on hemp straw residues to assess the effective performance of this feedstock in biogas production by reproducing the real operating conditions of an industrial plant. An organic loading rate was applied according to two different amounts of hemp straw residues (3% wt/wt and 5% wt/wt). Also, specific bioenhancers were used to maximize biogas production. When an enzymatic treatment was not applied, a higher amount of hemp straw residues determined an increase of the median values of the gas production rate of biogas of 92.1%. This reached 116.6% when bioenhancers were applied. The increase of the specific gas production of biogas due to an increment of the organic loading rate (5% wt/wt) was +77.9% without enzymatic treatment and it was +129.8% when enzymes were used. The best management of the biodigester was found in the combination of higher values of hemp straw residues coupled with the enzymatic treatment, reaching 0.248 $Nm^3 \cdot kg_{volatile\ solids}^{-1}$ of specific biogas production. Comparisons were made between the biogas performance obtained within the present study and those found in the literature review coming from studies on a laboratory scale, as well as those related to the most common energy crops. The hemp straw performance was similar to those provided by previous studies on a laboratory scale. Values reported in the literature for other lignocellulosic crops are close to those of this work. Based on the findings, biogas production can be improved by using bioenhancers. Results suggest an integration of industrial hemp straw residues as complementary biomass for cleaner production and to contribute to the fight against climate change.

Keywords: renewable energy technologies; sustainability; clean energy; bioenergy; biogas; industrial hemp; anaerobic digestion

1. Introduction

Industrial hemp (*Cannabis sativa* L.) is a valuable crop, and all parts of the plant can be used in many ways. Recent surveys carried out in the past few years (e.g., [1,2]) suggested that industrial hemp is a niche crop of increasing interest for its properties and versatility. New uses and innovative products appear on the market (more than 25,000 products have been discerned [3]), thus *Cannabis sativa* L. is becoming a very attractive crop on a global scale.

In Europe, hemp cultivation is mainly a multi-purpose crop. The market interest for hemp seeds and the need for attaining maximum economic viability of the related supply chains are stimulating a progressive shift of interest from traditional stem fiber use (textile, pulp or paper) towards multi-purpose cultivations. Indeed, in recent years, an increasing interest for new products obtainable as food or feed from seeds and for phyto-based cosmetics from inflorescence is emerging [4].

Hemp is a crop with fast growth, high biomass production at low inputs (fertilisers/pesticides), good CO_2 capture per hectare (about 2.5 t/ha), and soil protection due to the length of its roots, suitable for many industrial processes [5,6]. Appropriate soils for hemp are deep, show pH between 6.0 and 7.5, and have a good availability of nutrients and water holding capacity [7]. Moreover, hemp requires proper preparation of the seedbed, especially on clay soils, for a homogenous emergence due to its particular sensitivity to waterlogging. Sandy soils are less suitable for this cultivation, because of its poor water holding capacity determining greater water requirements [8]. It depends on climatic conditions. Indeed, in the South Mediterranean environment, higher irrigation volumes are required, with respect to the North Mediterranean one [9,10], but hemp water requirements are lower [11] compared to other specialized and common crops, such as maize, which are also cultivated for biogas production in Europe.

Industrial hemp cultivation is growing over time. A great increase was recorded from 2013 to 2017 in Europe [2], because of the introduction of policies and local incentives to the hemp industry [12].

As a result of the Italian Regulation [13], industrial hemp cultivation and processing assumed an increased national relevance. The regulation supports (also by including economic incentives) and promotes the development of integrated supply chains valuing research findings and pursuing local integration, as well as effective environmental and economic sustainability.

During the first decades of the 20th century, Italy was one of the most important producers on a global scale. In 1940, cultivated areas exceeded 100,000 ha, corresponding to more than 80,000 tons of hemp fibers [14].

The extension of the cultivated areas in Italy from 1961 to 2017 are reported in Figure 1 (sources of data: [15–17]).

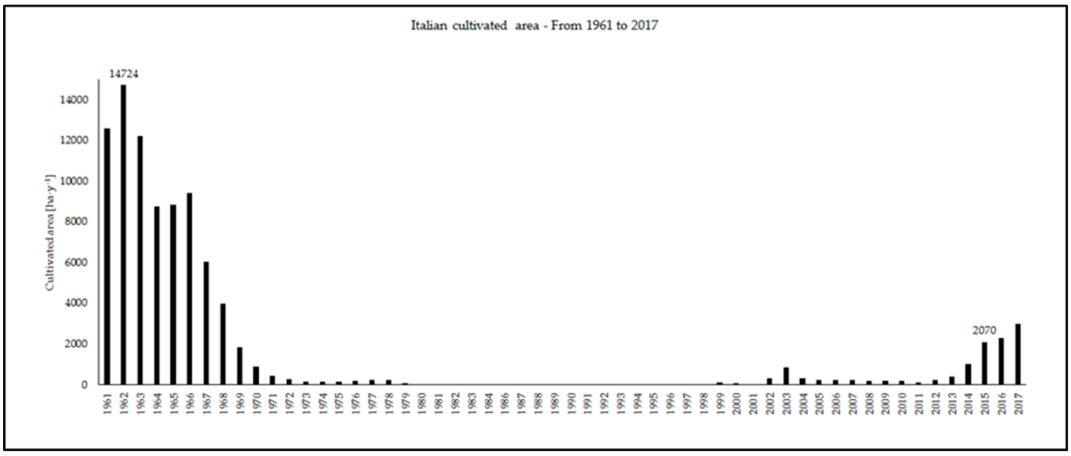


Figure 1. Hemp harvested areas (hectares by year) in Italy, from 1961 to 2017 (source of data: [15–17]).

Cultivated areas were significant during the 1960s and 1970s, and cultivations stopped in the 1980s and 1990s, mainly due to strict policies and regulations against the use of narcotic and psychotropic drugs. From 1999 to 2018, a new interest in hemp cultivation was developed, supported by national and European funding (the Italian Ministry of Agricultural Food and Forestry Policies financed a project to promote industrial hemp supply chains; during the same period, the European Union funded 3-year projects to reintroduce this feedstock for multiple purposes and to differentiate crops).

Due to the global resurgence of hemp cultivation needed to meet the requirements of the hemp sectors widespread today (building construction, food/animal feed, pharmaceutical, paper, textile, etc.), the recovery of hemp fiber and hurd residues should be addressed.

In this regard, some research and development projects funded by the European Union, such as MultiHemp [18] and GRACE [19], were already developed to demonstrate the sustainability of hemp-derived products according to the biorefinery concept.

However, another possible recycling path, aimed at increasing the economic and environmental benefits in a circular economy perspective, is the conversion of the agro-industry by-products into energy carriers.

Despite its thousands of uses, hemp by-products' potential as an energy feedstock is yet to be examined in depth. To date, few works have identified industrial hemp as an energy crop (for instance, a potential energy crop to produce bioenergy in [20]; ethanol production in [21,22]; methyl ester production in [23]; pyrolysis feedstock in [24]; biomass for thermochemical processes in [5]; combustion in [25,26]; co-firing in coal and peat power stations in [27]; and gasification or co-firing in [28]).

A few scientific works related to industrial hemp as a potential energy crop for biogas production can be found in the literature, going from 1990 to 2014. Rehman et al. [29] give an overall perspective of using hemp as a bioenergy crop in Pakistan, including biogas production.

Kreuger et al. [30], Heiermann et al. [31], Adamovics et al. [32], Mallik et al. [33] and Kaiser [34] provided data from anaerobic digestion trials carried out on a laboratory scale (a few liters-capacity reactors) (Table 1). As a pre-treatment, hemp was ground to a few mm or powder size. Since the grinding size influences the digestion kinetics [35] and, more generally, particle size affects the hydrolytic phase of the biodegradation of lignocellulosic feedstock [36], this factor should be considered when analyzing and comparing studies based on a laboratory scale. However, fine grinding is not reasonably achievable and economically affordable in industrial applications processing huge amounts of biomass.

Table 1. Main experimental conditions and information related to the scientific works found in the literature on anaerobic digestion of hemp, based on a laboratory scale.

Experiment	Hemp Cultivar	Country	Thermal Conditions	HRT	Specific Biogas Yield	Specific Methane Yield	Methane Content
[30]	Futura75	Sweden	50 °C	30 days	-	234 ± 35 $m^3 \cdot t^{-1}$ VS (mean ± std.dev. [1])	-
[31]	Fedora19	Germany	35 °C	35 days	453 ÷ 567 $L_N \cdot kg^{-1}$ VS	259 ÷ 301 $L_N \cdot kg^{-1}$ VS [2]	53 ÷ 57 (%vol)
[32]	Futura75 (among other cultivars)	Latvia	38 ± 1 °C	53 days	0.357 ÷ 0.370 $L \cdot g^{-1}$ VS (coarse particles); 0.470 ÷ 0.530 $L \cdot g^{-1}$ VS (fine particles)	0.172 ÷ 0.185 $L \cdot g^{-1}$ VS (coarse particles); 0.240 ÷ 0.270 $L \cdot g^{-1}$ VS (fine particles)	-

[1] std.dev. means "standard deviation"; [2] N means "normal", VS means "volatile solids".

For the present work, the study of Mallik et al. [33] was excluded from the comparison reported in Table 1, because of a lack of information about biogas production/methane production performance. In [34], experiments conducted in a batch digester were presented, where industrial hemp was co-digested with other vegetable wastes and poultry litter. These three types of biomass, fed to 10-L reactors, had the same size (2 cm), which did not allow the authors to consider the relationships between chemical composition and size, and how they influenced anaerobic digestion. It was difficult to assign biogas/methane production performances to each biomass making up the admixtures (with attention to hemp) to make comparisons with the results obtained in the present study.

The present work goes beyond the past research approaches to anaerobic digestion of hemp. It focuses on assessing actual opportunities of a large-scale use of industrial hemp straw residues. Indeed, in the south of Italy, hemp is primarily cultivated for seed production while hemp straw residues are ordinarily left in the field, due to their scarce economic value as well as to the limited industrial interest and knowledge about this by-product. This study aimed to provide a more comprehensive knowledge of this residual lignocellulosic biomass.

The screening carried out on hemp straw residues for biogas generation completes the major gaps identified in the related state-of-the-art, by offering in-depth knowledge of the effective performance of *Cannabis sativa* L. residues in biogas production.

This work considers an alternative use of hemp straw residues with respect to the already developed market sectors (see, for instance, [29]) and, consequently, it suggests new market opportunities for hemp-derived products. The outcomes show the effective potential of developing a new supply chain, based on an emerging lignocellulosic crop for biofuel production. These aspects will be economically relevant both for farmers and contractors in biogas/biomethane sectors.

As pointed out by [37], lignocellulosic crops are not a common source of biomass for biogas production. The authors emphasize that the most significant constraints to hemicellulose/cellulose digestion are related to the lignin content, crystallinity of cellulose, and particle size. These limits may be reduced through optimization of the methodologies and technologies supporting biogas and subsequent biomethane production, for more sustainable use of crop residues for energy purposes. Among other techniques, the use of specific enzyme systems should be considered to reduce the lignocellulose's recalcitrance to anaerobic digestion. An in-depth presentation of the chemical and biological mechanisms between recalcitrant biomass and enzymes was provided by [38].

As reported by [39], commercial bioenhancers are not thoroughly characterized, but the positive results provided by the recent literature (+30% increase circa, as reported by [40]) related to biogas production from biomass with a complex lignocellulosic structure stimulate further applications and studies.

This work includes treatment with a commercial preparation of bioenhancers developed to improve biogas production through anaerobic digestion of cellulose and hemicellulose in lignocellulosic crops, like *Cannabis sativa* L. residues, to contribute to an advance in the field.

Additionally, by assessing the current state of industrial hemp usage and deployment, it emerged that a synergistic approach along the entire supply chain should be adopted, by integrating high-value components of hemp and other parts of the plant into a well-designed biorefinery, in order to support the local economy in a more sustainable way.

2. Materials and Methods

In 2017, an experimental hemp crop on the *Cannabis sativa* L. cultivar "Futura75" was carried out at San Giovanni Suergiu (pilot site located in the south-western side of the Sardinian Island, Italy). This trial is part of the CANOPAES project (the Italian acronym for "CANapa: OPportunità Ambientali ed Economiche in Sardegna", focused on the environmental and economic opportunities of hemp in the Sardinian Island).

Futura75 was chosen for its diffusion in Europe and its ability to produce both seeds and biomass.

According to the available long-term data, the San Giovanni Suergiu's climate is typically the Mediterranean. During the crop cycle, the thermopluviometric trend was characterized by maximum temperatures above the average, and rainfall was equal to about one-third of the seasonal average. Hemp was sown at a density of 120 plants·m^{-2} and at a depth of 0.02 m.

In addition, 60 kg·ha^{-1} as urea were top-dressed at about one month after emergence. Irrigation was performed by sprinklers with 75% ETm (maximum evapotranspiration) restitution and no weed control was required. Hempseeds were harvested by an ordinary combine. After that, the by-product straw naturally dried on the field (moisture <15% on a wet basis). Then, straw was raked and baled for transportation to the pilot plant. The green biomass yield was about 20 t·ha^{-1} while naturally dried straw was about 3.7 t·ha^{-1}.

Based on the assumption that different uses of hemp straw can coexist, though the specific features of the local market drive the types of use (as stated by [6] and [7], the dual-purpose oil-fiber of *Cannabis sativa* L. is dominant in the European territory), this work assumed a hypothetical scenario made of a dual-purpose supply chain: Hempseeds were harvested by a combine, to be used for oil extraction, while residues were processed for energy carrier generation (specifically, biogas).

Then, single-step digestion was performed in the pilot plant described below. The duration of the experiment was of 423 days (from March 2018 to June 2019).

2.1. Feedstock Characterization and Pre-Treatment, Admixture Preparation, and Pilot Plant

Since the chemical composition and physical characteristics (e.g., moisture content M) were used to define the admixtures proportion, to manage the process stability and to optimize anaerobic digestion, proximate analysis and ultimate analysis of hemp straw residues were performed.

Samples were prepared by drying hemp straw at 105 ± 2 °C in a thermostatic oven (Memmert GmbH, Schwabach, Germany), and by shredding and mixing the material through a cutter.

The proximate analysis was conducted using a thermogravimetric analyzer (TGA701, LECO Corporation, St. Joseph, MI, USA) following [41], to determine the moisture content (M), volatile solids on a dry basis ($VS_{d.b.}$), ash content, and fixed carbon (FC) (reported as percentage by mass [%wt]).

Total carbon, hydrogen, total nitrogen, and sulphur were determined by conducting the ultimate analysis through a CHNS analyzer (Truspec, LECO Corporation, St. Joseph, MI, USA) in accordance with [42].

Fiber composition (ADF: Acid detergent fiber, NDF: Neutral detergent fiber, ADL: Acid detergent lignin) of the lignocellulosic feedstock was used to determine the daily intake of enzymes (see Section 2.2). Values were obtained by using a fiber analyzer ANKOM 2000 (ANKOM, Macedon NY, USA), following the Van Sœst methodology [43–46]. Concerning the hemicellulose and cellulose contents, those values were estimated by subtracting ADF from NDF and ADL from ADF [47,48].

Chemical and physical characteristics (with their standard deviations) of hemp residues are listed in Tables 2–4.

Table 2. Proximate analysis of hemp residues. The cultivar "Futura 75".

Proximate Analysis	
	[%wt]
$M^1_{d.b.}$	7.71 ± 0.01
$VS^2_{d.b.}$	81.37 ± 0.08
$Ash_{d.b.}$	2.50 ± 0.25
$FC^3_{d.b.}$	16.13 ± 0.35

[1] M means "moisture"; [2] VS means "Volatile Solids"; [3] FC means "Fixed Carbon".

Table 3. Ultimate analysis of hemp residues. The cultivar "Futura 75".

Ultimate Analysis	
	[%wt]
$Carbon_{d.b.}$	47.41 ± 0.04
$Hydrogen_{d.b.}$	6.52 ± 0.10
$Nitrogen_{d.b.}$	1.64 ± 0.02
$Sulphur_{d.b.}$	0.18 ± 0.00

Table 4. Fiber composition (mean values, [% dry matter]) of hemp residues. The cultivar "Futura 75".

Chopped Hemp, Reproductive Stage		
ADL [1] [%wt]	**NDF [2] [%wt]**	**ADF [3] [%wt]**
7.87	59.16	44.40

[1] ADL means "Acid detergent lignin"; [2] NDF means "neutral detergent fiber; [3] ADF means "acid detergent fiber"

The feedstock characterization did not include parameters, such as starch and sugar contents, because of the composition of hemp straw residues mainly characterized by the lignocellulosic structure.

The anaerobic digester used in the present work was a tubular, horizontal reactor of 1.13 m^3 total volume. It is 2.25 m long and its external diameter is 779 mm. It was radially mixed using a mechanical stirrer. The reactor was fed via a pneumatic pump, conveying the substrate previously introduced into a 250-kg-capacity feeding hopper. The reactor was tested by filling it with 960 L of digestate (corresponding to about 85% of the total volume).

The digestate produced during the process was discharged into a 200-kg-capacity tank, by using a pneumatic pump. The reactor was heated by three electrical resistances located in its center, loading, and discharging sides.

Sampling operations for the reactor sludge were performed using two valves located in the loading and discharging sides of the reactor.

Operations and parameters settings were managed and controlled by a programmable logic controller (PLC).

The feedstock pre-treatment consisted in mechanical milling, by shredding hemp straw residues using a 20-L-capacity cutter (dry cut). Then, coarse particles (maximum size: 1 cm) were mixed with the recirculated digestate in a 40-L-capacity cutter. When necessary, different amounts of water were added.

The operative settings were changed during the experimental period to investigate different process conditions (see Section 2.2).

2.2. Feeding Phases

The reactor was filled with digestate coming from an anaerobic digestion industrial plant treating corn silage and triticale. The digestate was used as received from the industrial plant. Then, a start-up phase was performed. During this phase, the temperature was increased by 2 °C daily until a constant value of 39 °C (mesophilic conditions) was reached.

Subsequently, the daily feeding rate of admixtures (Q, [$m^3_{substrate} \cdot d^{-1}$]) was increased during the first phase to reach an adaptation of the bacterial consortium to the specific substrate. After the start-up phase, the hemp to digestate proportion (hereinafter: percentage of new hemp straw in the admixture C, [% wt/wt]), Q, and digestate recirculation ratio (R, adimensional) were changed during the experimental period.

C and R values were chosen keeping in account the fluid-dynamics behavior of the admixtures. An increase of the hemp straw amount, indeed, could make the pumping of the admixtures itself difficult.

Two reference values of C (C1: 3% wt/wt; C2: 5% wt/wt) were set to evaluate the process. These conditions were tested by considering the presence/absence of specific bioenhancers. Treatments were randomly applied during the entire experiment.

Concerning the ranges of the organic loading rate (OLR, [$kg_{VS} \cdot (m^3_{reactor} \cdot d)^{-1}$]), hydraulic retention time (HRT, [d], dependent on Q), R, and C, different regimes were defined.

The abovementioned variables were analyzed along with the specific gas production (SGP, [$m^3_{biogas\ or\ methane} \cdot kg_{VS}{}^{-1}$]) and the gas production rate (GPR, [$m^3_{biogas\ or\ methane} \cdot (m^3_{reactor} \cdot d)^{-1}$]).

A commercial enzymatic preparation (Micropan Biogas ®from Eurovix, IT) was applied to reduce the current supply of hemp straw residues and to maximize biogas production at the same time. It is made of microbial enzymes containing cellulase, lipase, xylanase, active principles of *Fucus Laminariae*, algae *Lithothamnium calcareum*, natural nutrients/grow factors, selected yeast, mineral biological catalysts rich in oligo elements, and selected microorganisms (facultative anaerobic bacteria, such as: *Bacillus subtilis*, *Bacillus macerans*; strictly anaerobic bacteria genus *Methanobacterium*). The specific gravity was 0.8 t/m^3.

The daily intake was introduced into the reactor by dissolving the powder into water (1:4 wt/v).

The dosage was divided into two parts, depending on the fiber composition of lignocellulosic feedstock (Table 4), C, and Q. The daily intake was defined by multiplying the percentage of cellulose and hemicellulose of hemp residues (see Table 4) with the hemp mass in Q and a coefficient of 0.05, as suggested by the producers. Thus, 20 g per day were obtained.

The first enzymatic inoculum of the reactor sludge was calculated by dividing the working volume of the reactor by Q, and by multiplying this value with the daily intake (500 g of bioenhancers were introduced into the reactor).

2.3. Management of the Reactor and Process Stability

Both the process stability and reactor features were controlled and managed by using a set of parameters.

Management of the reactor: As regards the reactor, the following were considered:

- TS (total solids), $VS_{d.b.}$, determined via proximate analysis on a weekly/sub-weekly basis for the new admixture introduced into the feeding hopper, the material in the hopper/in the digestate tank, and the sludge inside the reactor;
- HRT [d], calculated as:

$$HRT = \frac{V}{Q}, \quad (1)$$

where V is the total digester volume [$m^3_{reactor}$] and Q is the daily feeding rate [$m^3_{substrate} \cdot d^{-1}$];
- OLR ([$kg_{VS} \cdot (m^3_{reactor} \cdot d)^{-1}$]), calculated as:

$$OLR = \frac{V \cdot S}{Q}, \quad (2)$$

where S is the VS concentration on a wet basis in the feeding admixtures [$\%wt_{w.b.}$].

Management of process stability: The process stability was monitored by considering the below listed parameters:

- pH and FOS/TAC ratio (volatile fatty acids content/buffer capacity) of the reactor sludge (daily measures by means of, respectively, a multi-parametric analyzer Orion Versa Star (ThermoScientific Inc., Waltham, MA, USA) and an automatic titrator T70 (Mettler Toledo International Inc., Columbus, OH, USA));
- Biogas production [$m^3_{biogas} \cdot d^{-1}$] (daily values provided by a biogas flow meter);
- Biogas composition daily (CH_4, CO_2, O_2 [%wt]; NH_3, and H_2S [ppm]), determined using a portable gas analyzer GA2000 (Geotechnical Instruments UK Ltd., Coventry, UK). Biogas composition is strictly related to SGP and GPR;
- Temperature of the reactor sludge, measured through three temperature probes located in the center, the loading, and discharging sides of the reactor, and monitored through the PLC.

Performance parameters: The two main performance parameters considered in the anaerobic digestion trials carried out on hemp straw residues are:

- SGP [$Nm^3_{biogas\ or\ methane} \cdot kg_{VS}^{-1}$], calculated as:

$$SGP = \frac{G}{Q \cdot S}, \quad (3)$$

where Q and S were already described, G is the daily production of biogas/methane [$m^3_{biogas\ or\ methane} \cdot d^{-1}$];
- GPR ([$Nm^3_{biogas\ or\ methane} \cdot (m^3_{reactor} \cdot d)^{-1}$]), calculated as the daily production of biogas/methane per m^3 of sludge accumulated in the reactor.

The abovementioned parameters are related to:

- C (percentage of new hemp in the admixture, [% wt/wt]), calculated as:

$$C = \frac{Mass_{hemp}}{Mass_{hemp} + Mass_{digestate} + Mass_{water}} \cdot 100, \quad (4)$$

where: $Mass_{hemp}$, $Mass_{digestate}$, and $Mass_{water}$ are the mass of the hemp, digestate, and water composing the admixtures;

- R (digestate recirculation ratio, adimentional), following Equation (5):

$$R = \frac{\sum_i Mass_{digestate}}{\sum_i Mass_{hemp}}, \quad (5)$$

where $\Sigma_i Mass_{hemp}$ and $\Sigma_i Mass_{digestate}$ [g·10^3] are the cumulative amounts of new hemp or digestate composing hemp-digestate admixtures in a specific time window.

2.4. Statistical Analyses

Statistical analyses were performed on biogas composition, SGP, and GPR using Statgraphic Centurion XVI [49]. The mean, standard deviation, maximum and minimum values, and median were calculated for each feeding phase composing the experimental period. Also, the skewness and kurtosis were calculated to determine which kind of statistical analysis should be performed.

Depending on this first data analysis and with a specific focus on the skewness and kurtosis, any evenness emerged among variances. Consequently, non-parametric tests were applied.

Two non-parametric tests were considered: Mann–Whitney [50,51] and Kruskal–Wallis [52]. The former was used to determine if two ordinal and independent random samples (feeding phases) were part of the same population. The latter was useful to compare median values of the different groups to identify whether they belonged to a population characterized by the same median.

Statistical analyses were developed by considering $p < 0.05$. Graphical representations were provided by using box-and-whisker diagrams.

3. Results and Discussion

3.1. Feeding Phases

Based on the outcomes of the experiment, it was divided into phases. The main characteristics of the feeding phases, except for the start-up phase, are reported in Table 5. Indeed, the start-up phase was characterized by high instability of the main process parameters as well as by a rapid variation of admixture feeding rates over time. Thus, the remaining feeding phases were labeled from 1 to 7.

Table 5. Main parameters (with their standard deviations) of anaerobic digestion trials on hemp residues.

Phase [-]	Description [-]	Duration [d]	OLR [1] [$kg_{VS}·m^{-3}·d^{-1}$]	HRT [2] [d]	C [3] [% wt/wt]	R [4] [-]
1	No enzymatic treatment	45 (day 98–day 143)	2.8 ± 0.6	29 ± 2	2.3 ± 0.0	16.3 ± 1.1
2	No enzymatic treatment	27 (day 144–day 171)	1.3 ± 0.2	34 ± 4	3.0 ± 0.9	18.9 ± 0.4
3	No enzymatic treatment	55 (day 172–day 227)	2.9 ± 0.8	34 ± 7	2.9 ± 1.8	20.7 ± 0.8
4	Enzymatic treatment	34 (day 228–day 262)	3.8 ± 0.8	31 ± 7	2.5 ± 1.7	22.0 ± 0.3
5	Enzymatic treatment	35 (day 263–day 298)	3.2 ± 0.8	30 ± 6	5.1 ± 0.2	22.0 ± 0.4
6	No enzymatic treatment	36 (day 299–day 335)	3.1 ± 0.9	33 ± 9	5.2 ± 1.0	20.9 ± 0.3
7	Enzymatic treatment	87 (day 336–day 423)	3.1 ± 1.0	29 ± 3	4.4 ± 2.0	20.3 ± 0.2

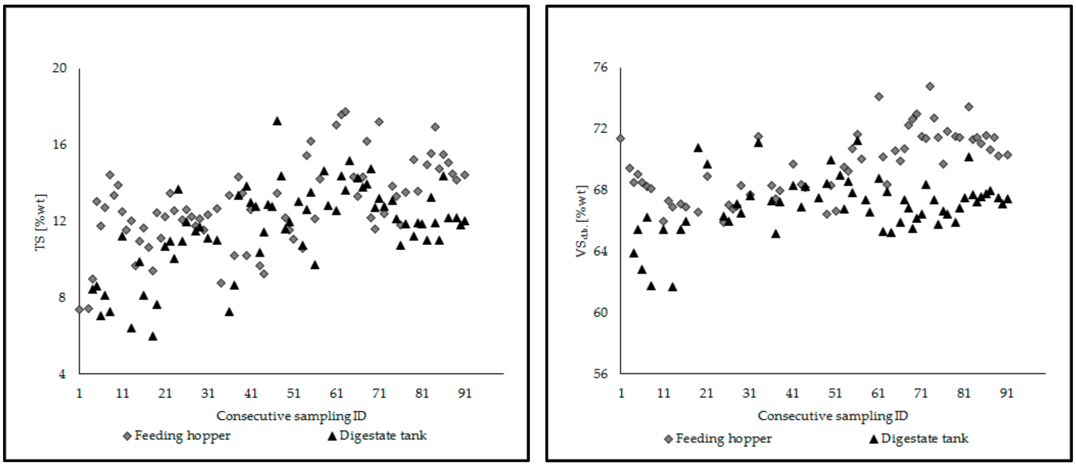
[1] OLR means "organic loading rate"; [2] HRT means "hydraulic retention time"; [3] C means "percentage of new hemp in the admixture"; [4] R means "digestate recirculation ratio".

Feeding phases were classified according to enzymatic treatment, OLR, HRT, C, and R (Table 5).

The two C reference values applied in this study are close to the organic loadings used in the industrial plants of anaerobic digestion.

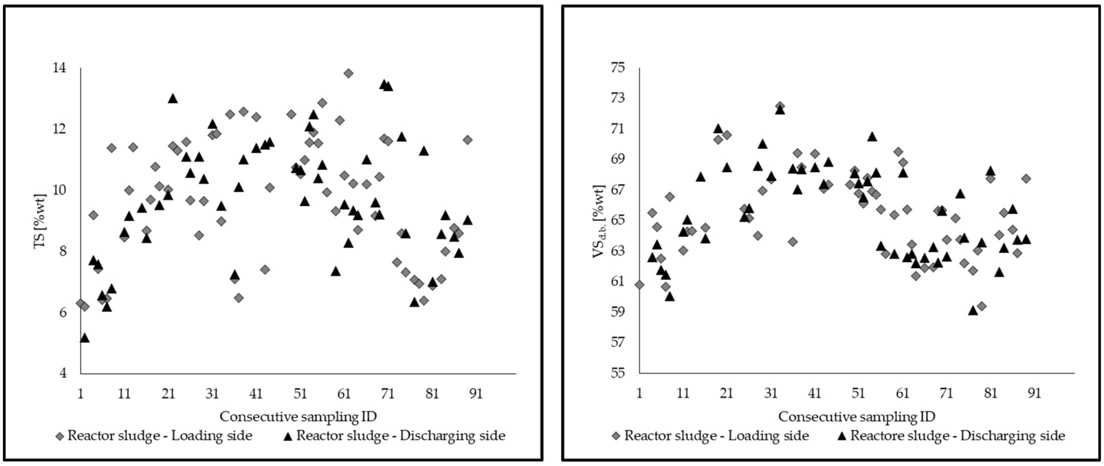
3.2. Management of the Reactor and Process Stability

Management of the reactor -TS and $VS_{d.b.}$ trends of the material in the feeding hopper, in the reactor sludge, and the digestate tank are reported in Figures 2 and 3.



a) b)

Figure 2. TS (total solids) (**a**) and $VS_{d.b.}$ (volatile solids on a dry basis); (**b**) trends of the material in the feeding hopper and the digestate tank, concerning consecutive sampling.



a) b)

Figure 3. TS (total solids) (**a**) and $VS_{d.b.}$ (volatile solids on a dry basis); (**b**) trends of the reactor sludge, concerning consecutive sampling.

By comparing the TS and $VS_{d.b.}$ trends in the feeding hopper and digestate, it can be seen that from the 70th sampling, these parameters were notably lower in the discharged sludge than in the corresponding feeding mixtures. The 70th sampling corresponds to the beginning of the 5th feeding phase and this occurrence emerged from the observations related to the 6th and 7th phases as well. In the previous feeding phases, a distinction between the TS and $VS_{d.b.}$ evolution in the fed slurry and the corresponding digestate cannot be seen. This result is related to the higher reference value of C (C2), which was better than the other one (C1).

With regard to Figure 3, the reactor sludge did not show relevant differences in terms of TS and $VS_{d.b.}$ by comparing the loading side to the discharging side, mainly due to a certain mixing of the sludge along the longitudinal section of the reactor. TS and $VS_{d.b.}$ seemed to increase from phase "1" to phase "4" and to decrease in the subsequent phases (characterized by the reference value, C2).

Management of process stability: Concerning the main design and operation process parameters of the reactor, the trends of HRT and OLR are shown in Figures 4 and 5.

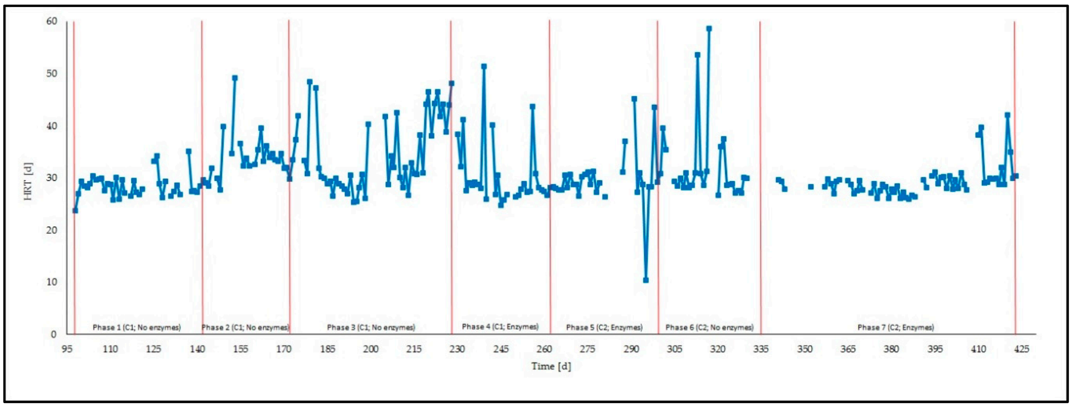


Figure 4. Hydraulic retention time (HRT) trend.

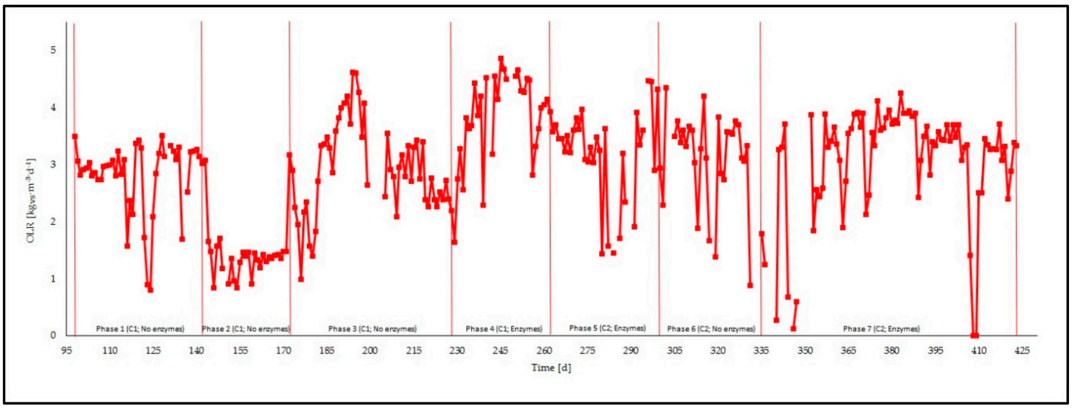


Figure 5. Organic loading rate (OLR) trend.

HRT did not show significant variations during the entire experiment (Figure 4) and it was excluded from the statistical analysis.

OLR (Figure 5) was monitored through the daily determination of TS and $VS_{d.b.}$ in the feeding admixture and adjusted by setting the hemp share in the feeding admixture and the loading flow rate (Figure 2).

Regarding the process stability parameters, the trends of FOS/TAC and pH, biogas production, and biogas composition (CH_4, CO_2, NH_3, H_2S) are reported in Figures 6–9.

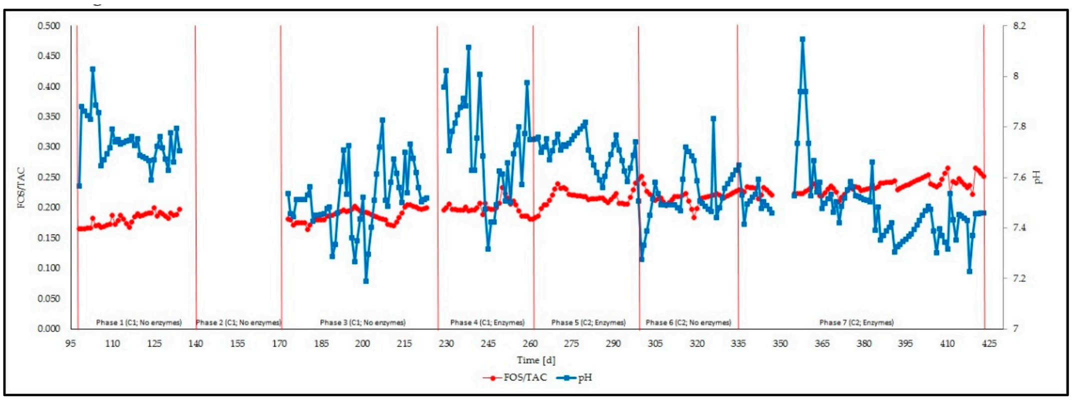


Figure 6. FOS/TAC (volatile fatty acids/buffer capacity) and pH trends.

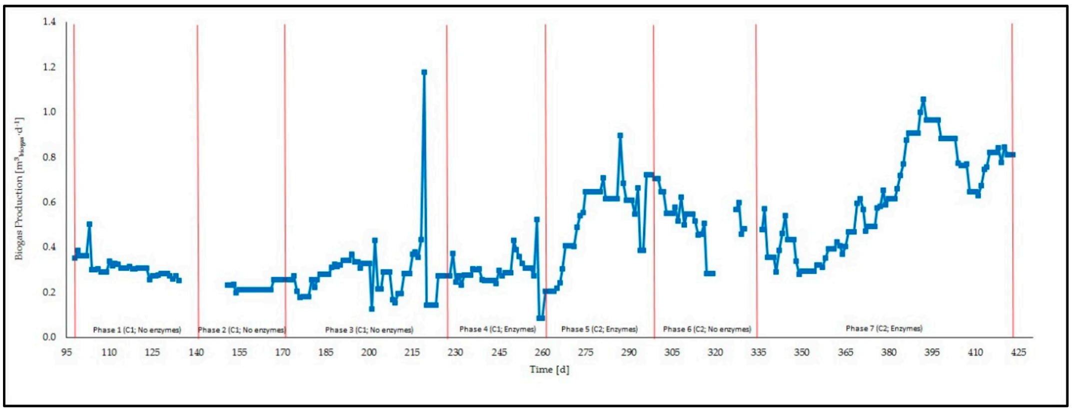


Figure 7. Biogas production trends (GPR) (via biogas metering).

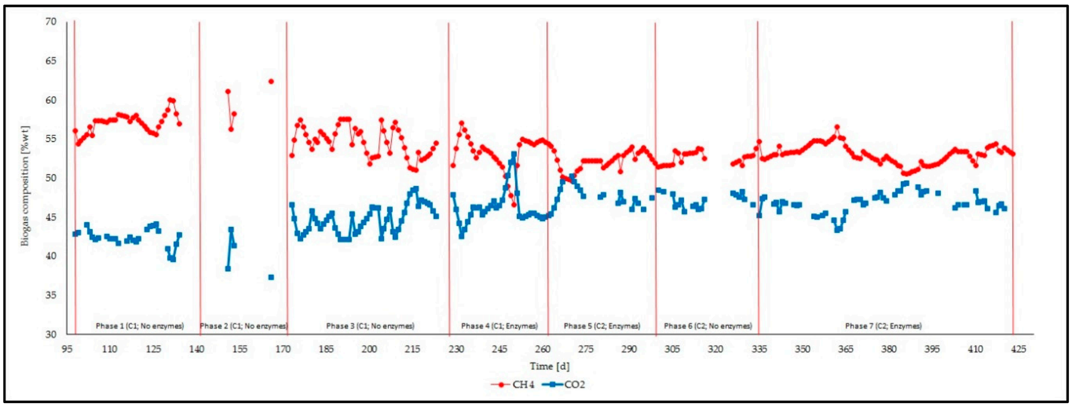


Figure 8. Biogas composition (CH_4 and CO_2, [%wt]) detected by the portable gas analyzer during the experimental period.

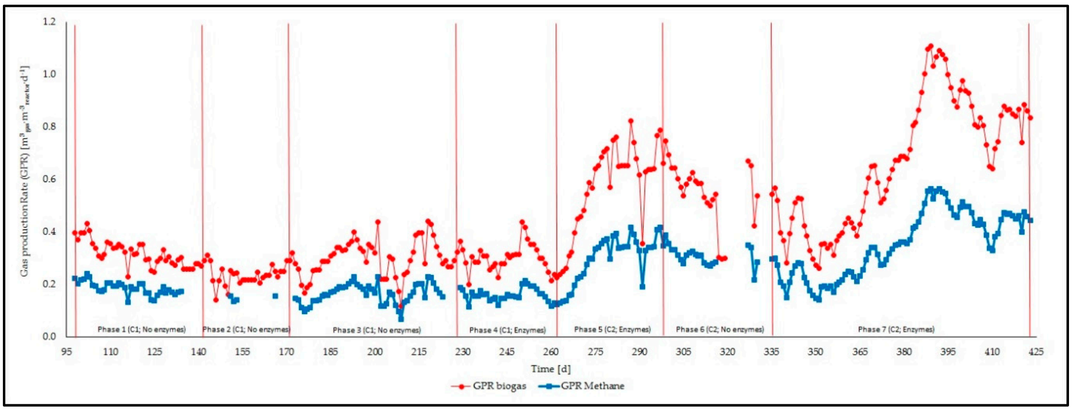


Figure 9. Gas production rate (GPR) trends of biogas and methane.

By considering the entire experimental campaign, pH varied between 7.2 and 8.0, accompanied by higher values during the first phase.

The FOS/TAC ratio increased over time, from about 0.170 to values close to 0.270. These values are very similar to the typical ones of the industrial plants treating lignocellulosic biomass, such as corn silage.

Overall, the FOS/TAC trend is consistent with the increase in the percentage of new hemp in the feeding admixtures (C2 treatment) (see Table 5) from the fifth feeding phase to the seventh one. It can be supposed that the introduction of higher amounts of hemp provoked a shift of the anaerobic digestion microbial dynamics towards the predominance of acidogenic reactions.

Daily biogas production (Figure 7) was characterized by high variability during the experimental campaign. It showed a significant increase of the biogas produced during the experimental phases from "5" to "7" (C2), accompanied by a rapid increase during the fifth phase and a decrease over the sixth one (without enzymes). The last feeding phase showed rising values for most of the days (to reach about 1.1 m^3 biogas per day), corresponding to the coupling of higher C and enzymatic treatment. It can be considered as the most suitable among all the treatments applied. On average, the CH_4 content [%wt] in the biogas produced during the entire experimental campaign was 53.8 ± 2.2. CO_2 content [%wt] was 45.6 ± 2.4. CH_4 concentration showed a slow reduction from the phase "1" to the phase "4". From the phase "5", a gradual increase of its concentration was detected. CH_4 and CO_2 trends (Figure 8) did not show any peak attributed to organic overload.

Performance parameters: The performance parameters, SGP and GPR, of the anaerobic digestion trials are shown in Figures 9 and 10.

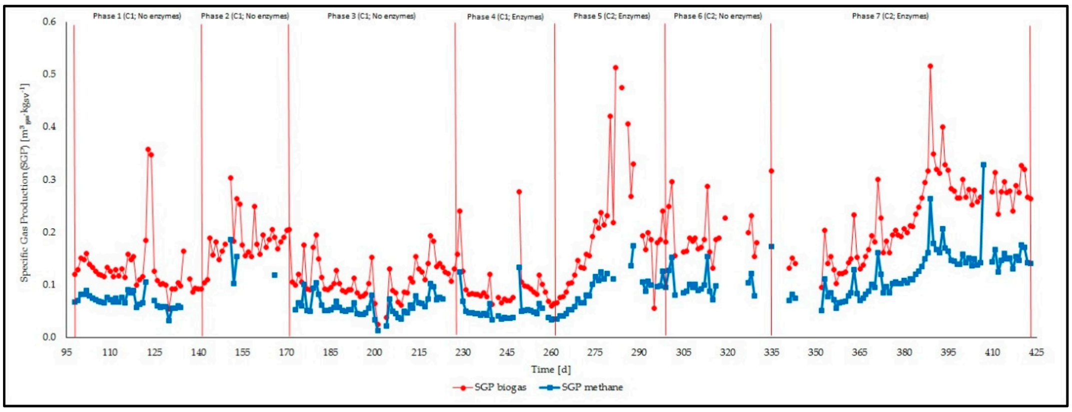


Figure 10. Specific gas production (SGP) trends of biogas and methane.

SGP of biogas/methane is the energy yield of an anaerobic digestion system regardless of OLR, thus it plays an important role when assessing the energy yield of the considered process.

Both trends showed an increase starting in the fifth feeding phase. It was more evident during the seventh period.

By comparing these outcomes with those obtained on a laboratory scale [30,32], it can be pointed out that the present work provided higher SGP values both for biogas and methane than the previous literature on anaerobic digestion trials of hemp straw. Overall, the management of the reactor and the process stability was enhanced with respect to biogas production, especially in the last part of the experiment, characterized by the combination of higher values of C and the application of enzymes.

SGPs of the present work are lower compared to those obtained in the same pilot plant, using vegetable feedstock characterized by high degradability (lower lignin contents and higher starch content). Due to the lignocellulosic nature of this crop, the specific biogas/methane production of hemp straw was lower than those related to raw potatoes (0.68 Nm^3 of biogas $\cdot kgVS^{-1}$ and 0.37 Nm^3 of methane $\cdot kgVS^{-1}$) [53], potato chips (0.81 Nm^3 of biogas $\cdot kgVS^{-1}$ and 0.47 Nm^3 of methane $\cdot kgVS^{-1}$) [54], and fruit and vegetable wastes (0.78 Nm^3 of biogas $\cdot kgVS^{-1}$ and 0.43 Nm^3 of methane $\cdot kgVS^{-1}$) [55].

Experiments carried out on the same pilot digester using admixtures made of different kinds of feedstock (admixture composition: 30%wt of shredded corn, the remaining part consisting in whey, vegetable water, pomace pitted, and manure to maintain the OLR between 2.5 and 3.5 $kgVS \cdot m^3{}_{reactor} \cdot d^{-1}$) reported SGP_{biogas} from 0.623 ± 0.212 $Nm^3 \cdot kgVS^{-1}$ to 0.768 ± 0.227 $Nm^3 \cdot kgVS^{-1}$ and $SGP_{methane}$ from 0.281 ± 0.160 $Nm^3 \cdot kgVS^{-1}$ to 0.438 ± 0.096 $Nm^3 \cdot kgVS^{-1}$ [56]. Also, hemp straw residues' performance in anaerobic digestion was found to be similar to other lignocellulosic crops [57], the specific methane production of which is between 0.17 and 0.39 $Nm^3 \cdot kgVS^{-1}$. Results are comparable to those reported by [58] for other agricultural crops, such as oats, flax, and sorghum, but lower than the hemp energy yields considered there.

Thus, hemp straw residues used in the same pilot plant showed lower SGP of biogas and methane probably because of the lignocellulosic composition. The results obtained by the residues considered in this work could be affected by the harvesting time, which is the reproductive stage when the lignin content is higher than in the vegetative stage. By considering that the reproductive stage is the core of the supply chain scenario assumed in this study, related to the extraction of oil from seeds, the results already discussed about the potential of biogas production from straw residues suggest the recovery of this low-value by-product to energy conversion. By considering the ultimate analysis, the carbon:nitrogen ratio is useful to define the biomass suitability for biochemical (ratio <30) or thermochemical processes (ratio >30). The ratio of hemp straw residues is 28.9 (Table 3), thus it can be considered for both, but with slightly higher suitability for biochemical conversion.

3.3. Statistical Analysis

Results of the data analysis (mean, standard deviation, maximum and minimum, skewness, kurtosis, and median values) carried out on the most significant process parameters and outcomes are reported in Tables 6 and 7.

Table 6. Main statistical variables of the biogas composition and biogas and methane gas production rate (GPR) and specific gas production (SGP).

Process Parameter	Variable	Unit	Feeding Phase	No. of Values	Mean ± Std.dev.	Minimum	Maximum
Biogas composition	CH_4	[%wt]	1	37	57.1 ± 1.2	54.4	60.0
			2	4	59.5 ± 2.8	56.3	62.4
			3	51	54.7 ± 1.9	51.0	57.6
			4	34	53.3 ± 2.3	46.6	57.1
			5	36	52.1 ± 1.2	49.7	54.1
			6	28	52.6 ± 0.9	51.4	54.7
			7	88	53.0 ± 1.2	50.5	56.6
	CO_2	[%wt]	1	27	42.4 ± 1.1	39.6	44.2
			2	3	41.1 ± 2.6	38.4	43.5
			3	51	44.7 ± 1.8	42.2	48.7
			4	34	46.2 ± 2.2	42.6	53.1
			5	20	47.7 ± 1.3	45.4	50.3
			6	19	47.0 ± 1.0	45.2	48.5
			7	52	46.8 ± 1.3	43.4	49.4
GPR	GPR Biogas	[$Nm^3 \cdot d^{-1}$]	1	46	0.317 ± 0.047	0.228	0.433
			2	28	0.232 ± 0.035	0.140	0.291
			3	56	0.299 ± 0.070	0.119	0.441
			4	35	0.301 ± 0.052	0.199	0.438
			5	36	0.581 ± 0.162	0.238	0.238
			6	26	0.552 ± 0.115	0.297	0.297
			7	88	0.668 ± 0.036	0.262	1.109
	GPR CH_4	[$Nm^3 \cdot d^{-1}$]	1	37	0.186 ± 0.025	0.132	0.241
			2	4	0.147 ± 0.010	0.135	0.155
			3	51	0.164 ± 0.038	0.068	0.231
			4	34	0.160 ± 0.025	0.114	0.215
			5	36	0.303 ± 0.086	0.129	0.418
			6	23	0.307 ± 0.036	0.218	0.388
			7	88	0.353 ± 0.123	0.141	0.424
SGP	SGP Biogas	[$Nm^3 \cdot kgVS^{-1}$]	1	45	0.129 ± 0.054	0.054	0.358
			2	27	0.191 ± 0.037	0.148	0.304
			3	54	0.110 ± 0.035	0.025	0.194
			4	33	0.097 ± 0.047	0.059	0.277
			5	32	0.207 ± 0.113	0.055	0.514
			6	23	0.198 ± 0.048	0.132	0.316
			7	75	0.250 ± 0.119	0.095	0.825
	SGP CH_4	[$Nm^3 \cdot kgVS^{-1}$]	1	35	0.069 ± 0.013	0.032	0.105
			2	4	0.140 ± 0.037	0.103	0.186
			3	49	0.060 ± 0.019	0.013	0.104
			4	31	0.050 ± 0.023	0.033	0.133
			5	27	0.092 ± 0.033	0.041	0.174
			6	20	0.104 ± 0.027	0.071	0.173
			7	75	0.132 ± 0.062	0.052	0.439

Table 7. Skewness, kurtosis, and median values of the biogas composition, and biogas and methane gas production rate (GPR) and specific gas production (SGP).

Process Parameter	Variable	Unit	Feeding Phase	No. of Values	Skewness	Kurtosis	Median
Biogas composition	CH_4	[%wt]	1	37	0.041	0.571	57.3
			2	4	−0.167	−1.191	–
			3	51	−0.297	−1.401	44.7
			4	34	−3.061	2.19	53.8
			5	36	−1.043	−0.722	52.2
			6	28	0.773	−0.717	52.5
			7	88	0.543	0.326	53.1
	CO_2	[%wt]	1	27	−1.652	1.25	42.3
			2	3	−0.383	–	–
			3	51	0.934	−1.069	43.6
			4	34	3.808	3.712	45.7
			5	20	0.478	−0.446	48.2
			6	19	−0.257	−0.962	46.6
			7	52	−1.431	0.653	46.8
GPR	GPR Biogas	[$Nm^3 \cdot d^{-1}$]	1	46	1.231	−0.350	0.311
			2	28	−1.027	1.151	0.232
			3	56	−0.473	−0.079	0.295
			4	35	1.028	0.835	0.303
			5	36	−2.012	−0.395	0.639
			6	26	−2.098	0.995	0.582
			7	88	0.161	−2.276	0.674
	GPR CH_4	[$Nm^3 \cdot d^{-1}$]	1	37	−0.197	0.073	0.188
			2	4	−0.444	−1.202	–
			3	51	−0.761	−0.559	0.168
			4	34	0.588	−0.33	0.157
			5	36	−1.944	−0.643	0.339
			6	23	−0.172	1.138	0.310
			7	88	0.000	−2.399	0.351
SGP	SGP Biogas	[$Nm^3 \cdot kgVS^{-1}$]	1	45	8.717	16.069	0.118
			2	27	3.400	2.619	0.182
			3	54	1.225	0.748	0.104
			4	33	6.655	9.930	0.084
			5	32	2.940	1.566	0.193
			6	23	2.462	0.869	0.185
			7	75	8.592	16.019	0.248
	SGP CH_4	[$Nm^3 \cdot kgVS^{-1}$]	1	35	0.240	1.828	0.069
			2	4	0.375	−0.860	–
			3	49	1.195	0.743	0.054
			4	31	6.431	9.402	0.046
			5	27	0.570	0.032	0.080
			6	20	2.450	0.980	0.095
			7	75	9.078	17.583	0.130

As already mentioned in Section 2.4, skewness and kurtosis indices show that, except for SGP of biogas, the considered parameters can be attributed to a normal distribution, but variances of the two groups differ. Thus, comparisons were made on medians instead of mean values.

The outcomes of the Kruskal–Wallis test performed on the feeding phases "3", "4", "5", and "6", which are the most representative phases with respect to the treatments applied in this work (C1 and C2; presence/absence of enzymes), are shown in Figure 11.

The main outcomes of the two comparisons, "3" and "6", and "4" and "5", are described below.

Methane content [%wt] showed a tendency to lower median values when C is higher (C2) and higher values for the lower C regime (C1). This is confirmed by the Kruskal–Wallis test. Conversely, the behavior of the median value of the CO_2 content in biogas is opposite to the methane content, and it was higher for the higher C regime (C2). This is due to the addition of new raw material that modifies the reactions towards acidogenic conditions and promotes an increase of the CO_2 content in biogas with respect to the effect of the digestate recirculated, containing an amount of undigested substrate that is less reactive.

An increase of C led to an increase of GPR (median values: Phase "3": 0.295 $Nm^3 \cdot d^{-1}$; phase "4": 0.303 $Nm^3 \cdot d^{-1}$; phase "5": 0.639 $Nm^3 \cdot d^{-1}$; phase "6": 0.582 $Nm^3 \cdot d^{-1}$). When the enzymatic treatment was not applied (phase "3" and phase "6"), the higher C regime (C2) corresponded to an increase of the GPR_{biogas} median values of 92.1%. This was about 116.6% when bioenhancers were applied.

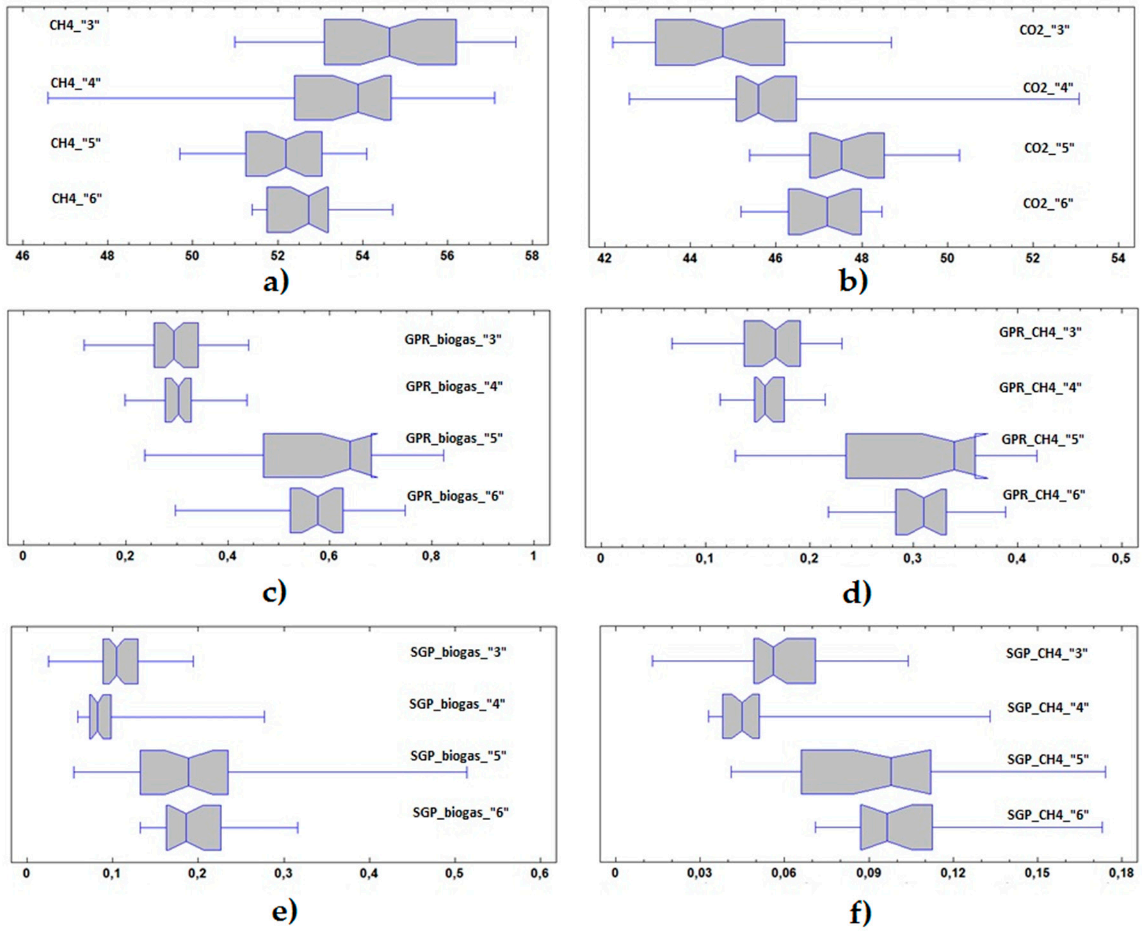


Figure 11. Box-and-whisker plots of the feeding-phases "3"–"6" and "4"–"5". Kruskal–Wallis test on the statistic variables: CH_4 content in biogas (**a**), CO_2 content in biogas (**b**), gas production rate (GPR) biogas (**c**), GPR methane (**d**), specific gas production (SGP) biogas (**e**) and SGP methane (**f**).

$GPR_{methane}$ showed similar trends observed for GPR_{biogas}: Higher values in the phases characterized by the C2 regime (median values: Phase "5": 0.339 $Nm^3{\cdot}d^{-1}$; phase "6": 0.310 $Nm^3{\cdot}d^{-1}$) than in those related to the C1 treatment (median values: Phase "3": 0.168 $Nm^3{\cdot}d^{-1}$; phase "4": 0.157 $Nm^3{\cdot}d^{-1}$). Hence, the increase due to the higher C regime was, respectively, +115.9% and +84.5% with and without enzymes.

With regard to the SGP of biogas/methane, an increase of C values led to higher energy yields.

The increase of SGP_{biogas} due to the increment of C was +77.9% without enzymatic treatment and +129.8% with enzymatic treatment. Thus, the coupling of a higher C regime with the addition of enzymes allowed the best management of the pilot plant to be obtained.

Essentially, similar behavior was observed for $SGP_{methane}$: The increasing of C promoted $SGP_{methane}$ (+165.9%) when enzymes were not applied. The increase associated with the enzymatic treatment was +73.9.

Statistically significant differences were found between phases "5" and "6" and phases "3" and "4", for all the parameters considered in the statistical analysis. Thus, it is reasonable to assert that variations of C influence all the parameters contributing to energy yields (SGP, GPR). The enzymatic treatment, instead, showed statistically significant differences in SGP in phases characterized by lower C values ("3" and "4").

The results of the Mann–Whitney test on the feeding phases "6" and "7", performed in order to assess the effect of the enzymatic treatment when the biodigester is managed by applying higher values of C, are reported in the box-and-whisker plot of Figure 12.

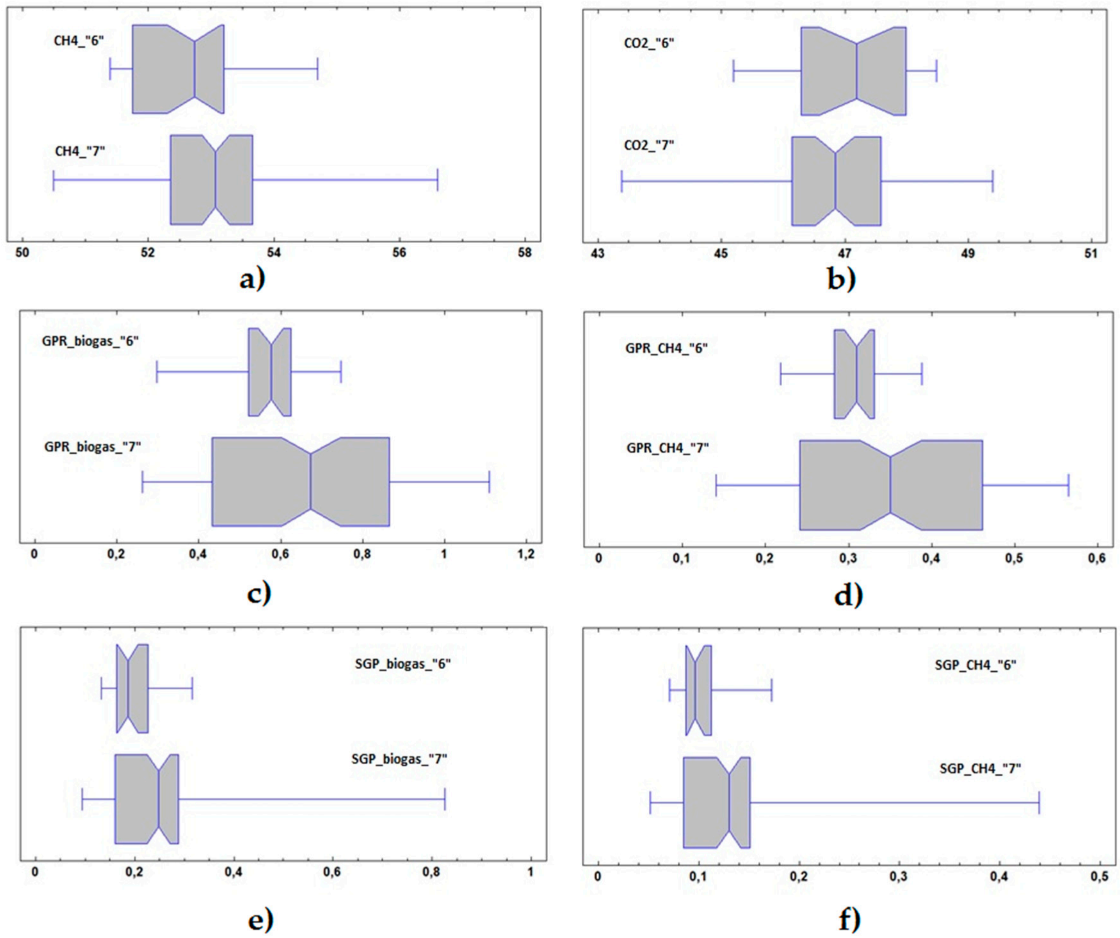


Figure 12. Box-and-whisker plots of the feeding-phases "6" and "7". Mann–Whitney test on the statistic variables: CH_4 content in biogas (**a**), CO_2 content in biogas (**b**), Gas Production Rate (GPR) biogas (**c**), GPR methane (**d**), Specific Gas Production (SGP) biogas (**e**) and SGP methane (**f**).

Skewness and kurtosis (Table 7) showed normal distributions of CH_4 and CO_2 contents in biogas, but SGP and GPR deviated from normality.

The Mann–Whitney test did not show any statistically significant difference in CH_4 content. Similar results were obtained for the CO_2 content in biogas (median of phase "6": 47.2% wt, median of phase "7": 46.8% wt).

SGP and GPR of biogas and methane were higher in the last phase of the experiment (with enzymatic treatment) than the second-last phase (without enzymes). More specifically, GPR and SGP of biogas and methane in the seventh phase reached the maximum values of the entire experimental campaign.

Concerning $GPR_{methane}$, any statistically significant difference was found between the two feeding phases (median value of phase "6": 0.310 $Nm^3 \cdot d^{-1}$, median value of phase "7": 0.351 $Nm^3 \cdot d^{-1}$).

More generally, in the last feeding phase, characterized by the enzymes, SGP and GPR of biogas and methane were higher than in the previous periods. Thus, the best management of the biodigester was characterized by this combination: A higher percentage of new hemp straw in the admixtures (C2) coupled with enzymatic addition.

4. Conclusions

The experimental campaign carried out on the cultivar "Futura 75" grown on the pilot site "San Giovanni Suergiu" (Sardinia, Italy) allowed an assessment of the effectiveness of using *Cannabis sativa* L. straw residues as a substrate for anaerobic digestion at an industrial scale and to enhance the management of the biodigester fed with hemp straw residues.

In this work, the feasibility of using this substrate in anaerobic digestion (which is currently often underutilized) was evaluated.

Results in terms of GPR and SGP of biogas/methane are promising, especially if compared to other vegetable feedstocks commonly used in anaerobic digestion and by considering that industrial hemp is characterized by higher values of lignin, which leads to high recalcitrance [59,60]. However, the SGP of biogas/methane is lower compared to corn silage, commonly used in industrial plants of anaerobic digestion (common values of about 0.7 to 0.85 $Nm^3 \cdot kg_{VS}^{-1}$), but the low cost of hemp straw residues and their behavior in the anaerobic digestion contribute to the definition of this by-product as a good process moderator when using other types of biomass leading easily to process instability.

The comparison between the findings of this work and the literature related to previous experiments carried out on a laboratory scale led to the assertion that biogas and methane yields provided by the trials carried out on hemp straw residues in the Sardinian pilot digester are similar or higher than those provided by the previous studies based on few liters-capacity reactors. It should be considered that differences in the energy yields reported may depend on environmental conditions (climate, soil type, crop management, etc.), the different cultivars used, hemp stage (vegetative versus reproductive stage), and hence, chemical and physical characteristics (such as TS, $VS_{d.b.}$, carbon:nitrogen ratio, etc.).

Results of SGP are close to those of other lignocellulosic crops but lower than those produced by highly degradable vegetable feedstocks studied through the same pilot plant. It suggests conducting additional experimental studies on hemp straws residues as a co-substrate in anaerobic digestion involving one or more easily digestible types of biomass.

Energy yields of anaerobic digestion carried out on hemp straw residues are influenced by different operating conditions: Increased feeding admixture composition (depending on C and R) produced a statistically significant increase in terms of methane content in biogas and of the parameters influencing GPR and SGP. Enzymatic treatments tended to enhance the SGP of biogas/methane.

The fluid dynamics of hemp-digestate admixtures play an important role in digestion kinetics, affected by solid–liquid separation and solid particles' tendency to sedimentation. Thus, further research should pay attention to this topic, to define relationships between the reactor and specific characteristics of admixtures.

These prodromal studies based on pilot-scale experiments on *Cannabis sativa* L. residues should be continued by analyzing more extensive conditions for factors inhibiting anaerobic digestion of hemp (e.g., heavy metals absorbed by roots, straw, leaves, and seeds during plant growth), and to different daily intakes of enzymatic preparations. Further investigations should pay attention to the enzymatic or other chemical additives' effects on energy yields (GPR, SGP, etc.).

The sustainability of hemp straw residues' biogas conversion should be evaluated as well, to define achievable costs and economic benefits. This hypothetical chain based on this emerging crop must be compared to the most commonly used energy feedstock. The main advantage in the energy conversion of hemp straws residues is to use a by-product of a cultivation carried out to obtain seeds as the main product: The consumption of water and fertilizers, however limited, is necessary to obtain seeds and no other input is spent, except for harvest and transport operations from the field to the plant.

In addition, with respect to the hemp-related supply chain, it has to be considered that relevant constraints to industrial hemp market development are fewer innovations in harvesting technologies and processes or processing facilities, as well as transportation/distribution issues (mainly due to the high low bulk density of this type of biomass) [61]. New research should overcome these current limits of industrial hemp exploitation and valorization to ensure more effective development of sustainable supply chains.

The results of this study produce a baseline to stimulate new perspectives of using hemp straw residues in the biogas sector and to inspire its consideration in the biorefinery thinking.

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Conflicts of Interest: The authors declare no conflict of interest.

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