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Hydrogen and methane production through two stage anaerobic digestion of straw residues

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ABSTRACT

Anaerobic digestion of agricultural waste can contribute to the European renewable energy needs. The 71% of the 20,000 anaerobic digestion plants in operation already uses these agro-waste as feedstock; part of these plants can be converted into two stage processes to produce hydrogen and methane in the same plant. Biomethane enriched in hydrogen can replace natural gas in grids while contributing to the sector decarbonisation. Straw is the most abundant agricultural residue (156 Mt/y) and its conventional final fate is uncontrolled soil disposal, landfilling, incineration or, in the best cases, composting. The present research work focuses on the fermentation of spent mushroom bed, an agricultural lignocellulosic byproduct, composed mainly from wheat straw. The substrate has been characterized and semi-continuous tests were performed evaluating the effect of the hydraulic retention time on hydrogen and volatile fatty acids production. It was found that all the tests confirmed the feasibility of the process even on this lignocellulosic substrate, and also, it was identified HRT 4.0 d as the best option to optimize the productivity of volatile fatty acids (17.09 gCODVFAs/(KgVS*d)), and HRT 6.0 d for hydrogen (7.98 LH_2/(KgVS*d)). The fermentation effluent was used in biomethanation potential tests to evaluate how this process affects a subsequent digestion phase, reporting an increase in the energetical feedstock exploitation up to 30%.

1. Introduction

According to the most recent data in the European Union (EU) there are 9.1 million agricultural companies (Eurostat, 2023a), which are responsible for the annual production of around 350 Mt of food products (Istat, 2023). These companies employ about 8.7 million workers (Eurostat, 2023b) and represents the 1.3% of the gross domestic product (GDP) of the EU (Eurostat, 2023c). Among the agricultural productions, the main representatives are wheat (129 Mt/y), corn (73 Mt/y) and barley (52 Mt/y) (Eurostat, 2023b). Livestock's activities are very abundant too, in 2021 there were about 142 million pigs, 76 million bovine, 60 million sheep and 6 million horses (Eurostat, 2023d, 2023e).

All these activities generate a large amount of agricultural residues every year (such as straw, manure, prunings), which represent a disposal cost and an environmental problem. Straw is the most abundant residue, and in particular for wheat, the most widely grown cereal in the EU, it reaches a value of 156 Mt/year (Reynolds and Braun, 2022; Tufail et al., 2021). Currently, wheat straw, and in general agricultural residues, are often not valorized in a circular economy system (Gontard et al., 2018): a part of them is still being dumped in landfills, burned, or employed in the compost production. The anaerobic digestion (AD) process turns out to be the ideal solution for these wastes because it leads to a decrease in carbon footprint, and more it permits the biogas production, finalized to the cogeneration of heat and power. In addition, the usage of this type of substrates assures the production of a second-generation fuel, in contrast to the non-virtuous use of more noble feedstock as food or energy crops that cause the Indirect Land Use Change (ILUC) (European Commission, 2012).

Currently, about 20,000 AD plants are already in operation in the EU, and most of them employ agrowaste as feedstock, specifically 71% (Deremince and Königsberger, 2017). Almost all these plants are working in a single-stage configuration for the production of methane. In order to improve the exploitation of the substrates and increase the value of the AD system, a part of these plants could be revamping according to two stage anaerobic digestion (TSAD) configuration (Bertasini et al., 2023). It would allow the simultaneous production of hydrogen and methane, both with higher yields than the traditional AD (Bolzonella et al., 2018). The TSAD is possible by the physical separation

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of the acidogenic and methanogenic phases in two different reactors under different operational conditions. Specifically, Volatile Fatty Acids (VFAs) and hydrogen can be produced in the first reactor, while a biogas rich in methane is obtained in the second one (Kabir et al., 2022; Micolucci et al., 2018; Rawoof et al., 2021; Zhu et al., 2022). Moreover, mixing hydrogen and biomethane from the process can allow the formation of biohythane, which have better combustion properties than methane and lower greenhouse gases emission (Bolzonella et al., 2018; Lay et al., 2020; Mozhiarasi et al., 2023).

In the scientific literature, there are several studies that reported the feasibility of the process from the lab to the pilot scale, some of which reached a hydrogen yield greater than 100 LH2/KgVS (Bertasini et al., 2023). But the majority of these TSAD experiences were limited on substrates with a low lignocellulosic content, such as the organic fraction of municipal solid waste (OFMSW), which were easier biodegradable by microbial biomass.

The main aim of this research is the investigation of the potential of a rich lignocellulosic substrate for producing hydrogen, VFAs and methane. In particular, the influence of different hydraulic retention time (HRT) on the main products from the AD has been studied. The substrate selected as representative for the lignocellulosic agricultural residues was the spent mushroom bed (SMB), that is composed mainly from wheat straw and horse manure, common feedstocks of the AD processes due to the favorable C/N ratio.

2. Materials and Methods

The influence of the HRT on the SMB was observed under semi-continuous tests having the aim to find the best operational conditions able to optimize the hydrogen and VFAs production during the dark fermentation phase. Then, further batch tests have been performed in order to evaluate an eventual improvement on the biomethanation potential (BMP) of the SMB, previously treated in the semi-continuous tests.

2.1. Substrates characterization

The SMB and the inoculum were provided by the agricultural company "Cooperativa Agricola Zootecnica La Torre", Isola della Scala (VR), Italy. The mixed microorganism inoculum was a 2 mm filtered agricultural digestate, i.e., the final solid-liquid output derived from an AD plant using bovine manure, chicken dung, wheat straw and corn residues as substrates.

The substrates' characterization was performed in triplicate on the same samples following the IRSA CNR Standard Methods (IRSA CNR, 2023), in terms of Total Solids (TS), total Volatile Solids (VS), Chemical Oxygen Demand (COD), Total Kjeldahl Nitrogen (TKN) and total ammonia nitrogen (TAN).

2.2. Semi-continuous tests

The HRT influence on the acidogenic fermentation of SMB were observed considering different values in a range of 1.5–18 days (Table 1). The semi-continuous tests were provided in 1 L "Duran" glass reactors with a working volume of 0.6 L, equipped with a Rushton impeller at 90 rpm. The feeding and the discharge of the reactors were performed manually one time per day, taking into account the different HRT (Table 1). The reaction medium was kept under a pH between 5.5 and 6.5, daily controlled with the addition of concentrated hydrochloric acid or sodium hydroxide, and a mesophilic temperature of 38 °C maintained through a thermostatic bath. The reactor was filled with the inoculum to ensure the microbial presence. The feeding of the reactor, composed by SMB and water, had a TS concentration of 5% w/w. This value was chosen considering the occurrence of problems in homogenization with a higher TS concentration. Due to the imposed 5% w/w in solids content, it was not possible to modify the Organic Loading Rate (OLR) values, which resulted 28.9 gCOD/(L*d), 10.8 gCOD/(L*d), 7.2 gCOD/(L*d), 6.2 gCOD/(L*d), 4.3 gCOD/(L*d), 3.6 gCOD/(L*d) and 2.4 gCOD/(L*d) following the ascending order of HRT values (Table 1).

Table 1
Semi-continuous tests reactor parameters in terms of HRT and OLR.

HRT d	OLR gCOD/(L*d)
1.5	28.89
4.0	10.83
6.0	7.22
7.0	6.19
10.0	4.33
12.0	3.61
18.0	2.41

The tests were daily monitored evaluating mainly the VFAs content and the gas production and composition. Solids, COD, nitrogen, and TAN were also checked to monitor the evolution of the system and the maintenance of an appropriate C/N ratio.

2.3. Analytical methods

The gas volume produced was quantified with a water displacement method and the qualitative analysis was carried out with a "Geotech Biogas 5000 analyzer". The analyzer can detect CH_4, CO_2, O_2 and remaining gas in volumetric percentages (with an accuracy of 0.5%), and H_2S in ppm. In particular, hydrogen content was calculated mathematically by difference.

The total VFAs concentration was verified through ion chromatographer "Thermo Fisher Scientific ICS-1100", from C1 to C6, and once the reactors reached the steady state, i.e., acclimatization of the microbial community has been achieved, were calculated the yield and the productivity for each trial. The VFAs yield and VFAs productivity were obtained following the Equation (1) and Equation (2), respectively.

$$VFAs\ Yield\left(\frac{gCOD\ VFAs}{KgVS}\right)=\frac{total\ \ VFAs\ produced}{total\ VS\ added\ as\ substrate} \tag{1}$$

$$VFAs\ Productivity\left(\frac{gCOD\ VFAs}{KgVS*d}\right)=\frac{total\ \ VFAs\ produced}{(total\ VS\ added\ as\ substrate\ *\ HRT)} \tag{2}$$

2.4. Evaluation of the kinetics of the acidogenic fermentation

The modified Stover-Kincannon model was used to extrapolate the parameters related to the substrate removal in dependence of the OLR value. This model is commonly used for anaerobic digestion process and it can be also applied to the fermentation stage (de la Lama et al., 2017; Nasr et al., 2015). The kinetic model is based on the expression of the Equation (1), that, plotting the HRT as abscissas axis and $\frac{HRT}{(So-Se)}$ as ordinates axis, assumes the format $y = mx + q$. In the Equation (3), So and Se are the substrate VS concentrations before and after the process, in terms of gVS/L.

$$\frac{HRT}{(So-Se)}=\frac{Kb}{(Rmax*So)}*HRT+\frac{1}{Rmax} \tag{3}$$

The kinetic parameters Kb and $Rmax$ are then calculated from the slope and intercept of the resulting trend line, respectively. Once obtained, are useful to predict the Se resulting at any HRT and So values, through the Equation (4).

$$Se=So-\frac{Rmax*So}{Kb+\left(\frac{So}{HRT}\right)} \tag{4}$$

2.5. Methanogenesis tests

After the fermentation tests, BMPs tests were processed on the SMB outputs from the semi-continuous tests to simulate the methanogenic phase of the TSAD. Furthermore, a BMP of the fresh substrate (i.e. not fermented) was performed (0 HRT test) in as a comparison to verify the "pretreatment effect" of the first phase (Micolucci et al., 2018). The BMP tests were prepared considering a working volume of 0.5 L, Substrate: Inoculum Ratio of 1:1 in VS terms and mesophilic condition (38 °C). The duration of the BMP was of 50 days. The biogas produced, likewise for the semi-continuous tests, was measured through water displacement method and qualitatively analyzed with a "Geotech Biogas 5000 analyser".

2.6. A basic energy comparison between the mono and the TSAD

In order to demonstrate the convenience of the TSAD, a basic energy balance was provided considering the amounts of the main gases produced from acidogenic fermentation (hydrogen) and from the methanogenic phase (methane) and their correspondent Lower Heat Value (LHV), which are 239.20 kJ/mol and 800.30 kJ/mol, respectively (Malave et al., 2014). Specifically, for hydrogen we considered the results from the best HRT (6 days) of the semi-continuous mode:

$$Produced\ hydrogen\left(\frac{mol}{d}\right) * HRT\ (d) * LHVH2\left(\frac{KJ}{mol}\right) = \mathrm{EH_2\ (KJ)} \tag{5}$$

While for methane from batch test we considered the methane production derived from the outputs of the acidogenic fermentation of the semi-continuous tests performed with a HRT of 6 days:

$$Produced\ methane\ (mol) * LHVCH4\left(\frac{KJ}{mol}\right) = \mathrm{ECH_4\ (KJ)} \tag{6}$$

The two energetical contributions were summed up and compared with the energy derived from the methane of the "0 HRT" test, which was used to simulate the mono stage AD of the SMB.

3. Results and discussion

3.1. Substrates characterization

The Table 2 shows the results and standard deviations of inoculum and feedstock characterizations.

The given values for the feedstock demonstrate a high TS content, while the COD and TKN values, deviate slightly from values found in literature, due to the usage of different SMB composition (such as wood chips, corn cobs and yard trimmings) (Gao et al., 2021; Lin et al., 2014). In particular, the presence of manure in the feedstock analyzed increases the nitrogen content, which comports a lower the C/N ratio of 14.4, value that is very close to the optimal dark fermentation range of 15–35 (Bertasini et al., 2023).

Table 2
Characterization of the agricultural digestate 2 mm filtered (inoculum) and SMB (feedstock).

	TS	VS	VS/TS	COD	TKN	TAN
	% w/w	% w/w	% w/w	gCOD/KgTS	gN/KgTS	gN/KgTS
Agricultural Digestate	4.1 ± 0.3	2.7 ± 0.0	65.9 ± 4.31	756.1 ± 9.6	292.8 ± 41.2	212.3 ± 4.8
SMB	29.2 ± 2.4	26.1 ± 2.4	89.4 ± 1.1	866.6 ± 12.7	60.2 ± 4.7	

3.2. Semi-continuous tests

Fig. 1 reported the evolution of the daily biogas production and of the measured pH. As reported above, each test was carried out for a period equivalent to 3 times the HRT value, and in all the cases, the steady state was achieved in a HRT and a half, as can be deduced from the maintenance of the constant pH value and volume of gas produced.

The Fig. 1 does not report the trial HRT 1.5 d, because the steady state was achieved in 2 days and the number of data collected was not sufficient to appreciate the difference between the acclimatation from the stationary phase. During each trial the TAN was also measured to prevent eventual inhibition of the process. The values measurements demonstrated that ammonia concentrations remained always lower than the inhibitory range of 1.5 gN/L and 7.0 gN/L (Rawoof et al., 2021). Therefore, microbial activity was not hindered during the process.

3.2.1. Gas production

During the process, it was evaluated the gas production in quantitative and qualitative terms (Table 3). Specifically, gas yields and gas productivities were calculated to assess which HRT values best fit with the energy recovery. The dark fermentation process, as citated previously, is able to produce mainly hydrogen and carbon dioxide, while the appearance of methane traces indicates that the processing is shifting towards the methanogenic phase, with the consequent consumption of hydrogen and VFAs. During the gas analysis, given the opening of the reactors for substrate insertion, traces of N_2 and rarely O_2 were also identified; these values were not reported individually but included in the gas total.

As reported from the values of methane yield of the table above, high HRTs promote the switch of the biological process from anaerobic fermentation to digestion, with methane formation passing from a HRT of 12.0 days–18.0 days. As expected, in fact, the longest HRT tested reached the highest methane yield with 17.92 LCH_4/KgVS (14% (v/v)). The best gas yield was obtained during test HRT 18.0 d, that reported the highest efficiency for the conversion of the organic matter in gas, 127.97 Lgas/KgVS. Considering the hydrogen yield, the best HRT was 10.0 d, which reported a value of 56.45 LH_2/KgVS. In light of this result, a similar value of 58.78 LH_2/KgVS was obtained in a work of dark fermentation of wheat straw in batch reactors using HRT of 16.0 d, which also integrated an alkaline pretreatment and a simultaneous saccharification fermentation (Reilly et al., 2014). Better results were obtained by Menzel et al. (2023), who worked with a different configuration of the process. They valorized a mixture of SMB (66% w/w) and maize silage in a plugflow reactor operating in the TSAD mode. They achieved an increase of the hydrogen yield of 256% (Menzel et al., 2023). Whereas, in terms of hydrogen productivity, HRT 6.0 d resulted the best with 7.98 LH_2/(KgVS*d), with an efficiency of feedstock conversion of 47.88 LH_2/KgVS.

3.2.2. VFAs production

The results of VFAs yields (gCOD/gVS) versus the applied HRT are shown in Fig. 2.

As reported above, the best VFAs yield was achieved during fermentation test with HRT 10.0 d, with 82.20 ± 10.63 gCODVFAs/KgVS. This value is larger than the conventional HRT used for the acidogenic fermentation, which are usually in the range of 0.5–3 days according to the complexity of the substrates (Bertasini et al., 2023). It can be explained taking into account the complexity of the chemical structure and the high molecular weight of the lignocellulosic polymers which compose the SMB. In this regard, wheat straw is comprised of 10–15% lignin, 30–35% hemicellulose and 35–40% cellulose (Tufail et al., 2021). As reported by Garcia et al. (2019), lignocellulosic feedstocks report hydrolysis constant rates around 0.08 d^{-1}, low values compared to the 0.15 d^{-1} of an energy crops. It is important to emphasize that higher HRT are recommended as they can avoid the

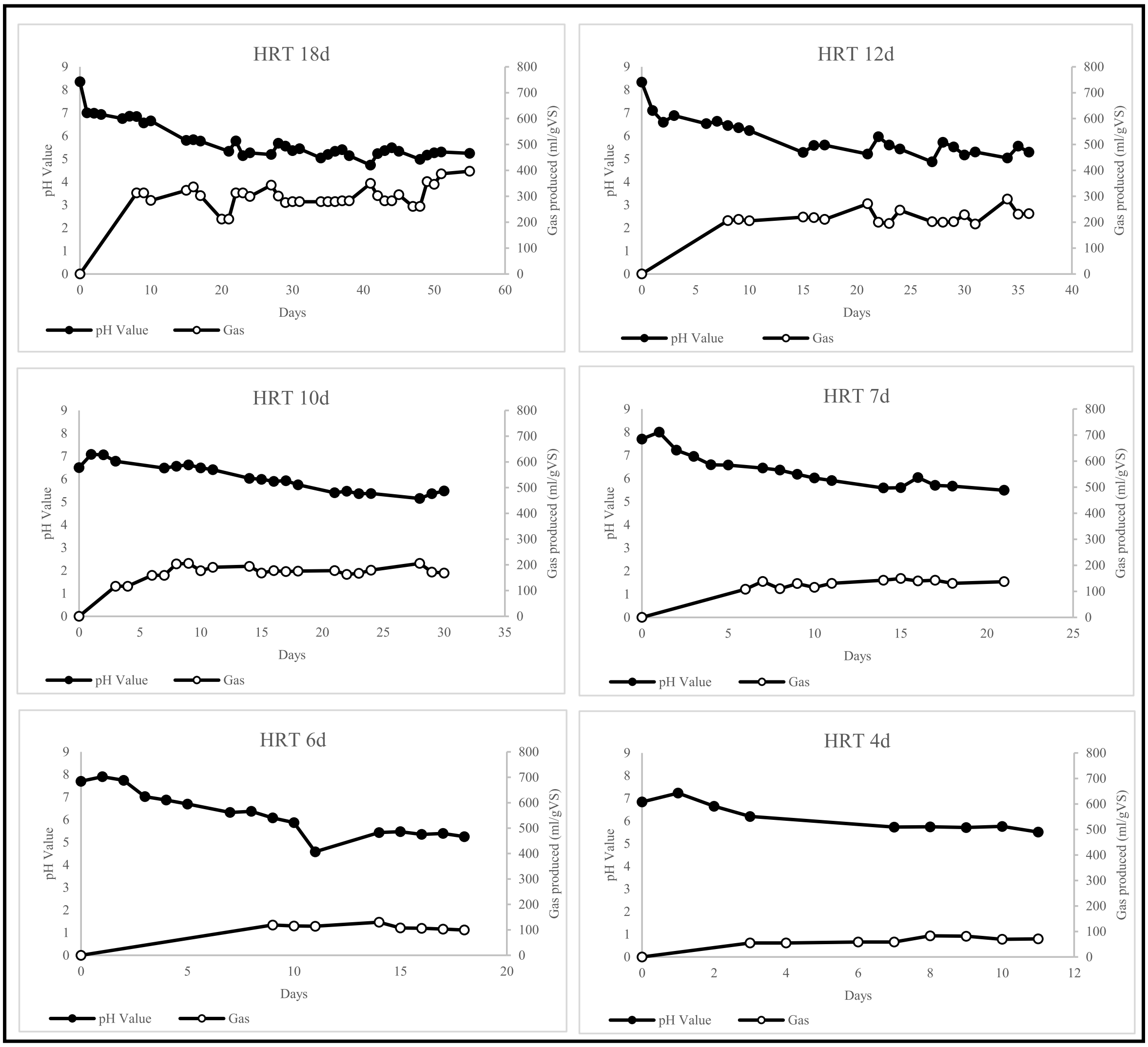

Fig. 1. Fermentation trends in terms of pH and gas produced at HRT value of 18.0 d, 12.0 d, 10.0 d, 7.0 d, 6.0 d and 4.0 d.

Table 3
Gaseous productivities and yields relating to each HRT tested.

HRT	Productivity		Yield			
	Total	H_2	Total	CH_4	CO_2	H_2
d	Lgas/ (KgVS*d)	LH_2/ (KgVS*d)	Lgas/ KgVS	LCH_4/ KgVS	LCO_2/ KgVS	LH_2/ KgVS
1.5	16.63	6.29	24.94	0.00	3.42	9.43
4	13.48	7.31	53.93	0.00	15.72	29.26
6	14.37	7.98	86.22	0.00	23.71	47.88
7	12.03	6.55	84.20	0.00	21.72	45.87
10	10.07	5.65	100.70	0.81	27.69	56.45
12	9.56	4.78	114.71	2.64	41.18	57.37
18	7.11	3.35	127.97	17.92	35.58	60.34

washout of the acidogenic microorganisms, but on the other side it can favoring the VFAs conversion into methane (Bertasini et al., 2023; Bolzonella et al., 2018; Menzel et al., 2023).

HRT 10.0 d and the C/N ratio adjustment given by the manure content could be the reasons for the high yield of VFAs compared with previous fermentation studies of wheat straw (Chen et al., 2012; Kabaivanova et al., 2022; Menzel et al., 2023; Reilly et al., 2014). Whereas in the case of HRTs 1.5 d and 18.0 d, the effect of C/N ratio did not play a major role during fermentation, reporting VFAs yields similar to the literature (Álvaro et al., 2023; Kabaivanova et al., 2022).

After the quantitative analysis, the effluent profile of the VFAs was also determined (Table 4).

Acetic acid, Propionic Acid and Butyric Acid were the main VFAs in all the tests performed at different HRT. In particular, the highest concentration of Acetic acid, 1.65 gCOD/L, was obtained in correspondence of the HRT 10.0 d, while Propionic Acid and Butyric Acid were maximised at HRT 6.0 d, with 1.08 gCOD/L and 0.93 gCOD/L, respectively. During this study, was produced mainly short chain VFAs, which are desirable products for the dark fermentation (Menzel et al., 2023) and find better use in a subsequently digestion phase, due to a quicker methane conversion (Sinaga et al., 2017). The results from this study seems to be consistent with previous works in the scientific literature: the VFAs produced from the acidogenic fermentation of wheat straw are usually acetic acid, butyric acid, propionic acid and iso-valeric acid, in descending order according to concentration (Álvaro et al., 2023; Chen et al., 2012, 2022; Kabaivanova et al., 2022; Menzel et al., 2023; Reilly et al., 2014; Zhu et al., 2023). The consistent production of propionic acid is unusual for this residue's fermentation, but may be accounted for by the presence of manure in the substrate, which is often traced to the formation of this VFAs resulting from lipids and proteins conversion (Coats et al., 2011; Tampio et al., 2019; Yin et al., 2022). The accumulation of propionic acid during fermentation can be an indication of a reduction in hydrogen yield. In fact, some microorganisms produce propionic acid from hydrogen (Bundhoo and Mohee, 2016), in addition, some hydrogen producers, need to change their metabolism to be able to

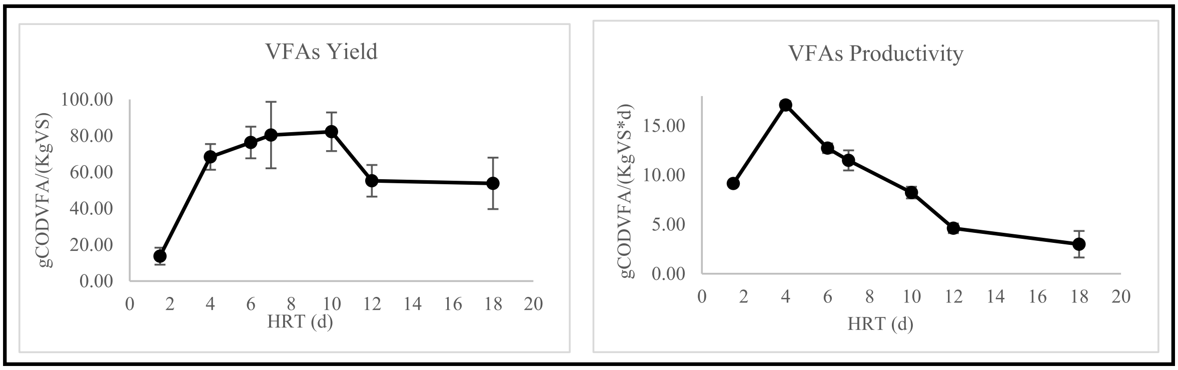

Fig. 2. Graphs of yield and productivity values in VFAs obtained during fermentation tests at different HRTs.

Table 4
Average VFAs concentrations obtained from each fermented output.

HRT	Formic	Acetic	Propionic	Iso-Butyric	Butyric	Iso-Valeric	Valeric
d	gCOD/L						
1.5	0.00	0.33	0.28	0.00	0.44	0.00	0.00
4.0	0.00	1.08	1.02	0.04	0.72	0.15	0.11
6.0	0.00	1.30	1.08	0.00	0.93	0.21	0.13
7.0	0.02	1.41	1.07	0.06	0.85	0.22	0.07
10.0	0.01	1.65	1.01	0.05	0.71	0.23	0.10
12.0	0.00	1.19	0.61	0.00	0.90	0.00	0.09
18.0	0.00	1.45	0.54	0.00	0.85	0.00	0.00

produce propionate (Hawkes et al., 2007; Tapia-Venegas et al., 2015; Wang et al., 2006). According to Sivagurunathan et al. (2014), the range of inhibition is wide and complex to define precisely, 0.2–12.0 g/L, as it is dependent on many factors such as substrate type, inoculum and feedstock. Therefore, it is difficult to define whether there were any inhibition events within the SMB trials. But at the same time, it is possible to rule out major inhibition events since the HRT 6 d trial reported both the highest hydrogen productivity and the highest propionic content.

3.2.3. Kinetic model

The modified Stoker-Kincannon model was applied to the VS data recovered during each steady state and was plotted below in Fig. 3. The trendline resulted has 0.0413 and 0.0479 as slope ($\frac{Kb}{Rmax*So}$) and intercept ($\frac{1}{Rmax}$), respectively.

Obtained these values, the kinetic parameters were calculated: 20.88 gVS/(L*d) as *Rmax* and 38.46 gVS/(L*d) as *Kb*. Consequently, it was possible to estimate the theorical substrate removal thanks to the Equation (4). The lacking biodegradable fraction corresponds to the products synthetized during the process, i.e., hydrogen, carbon dioxide and VFAs. The reliability of the built model was tested using all the HRT values examined, and the average deviation resulted was only 8%. de la Lama et al., 2017, reporting that a deviation lower than 15% is low and indicates the proper functioning of the built kinetic model, so all the more reason with a lower error value.

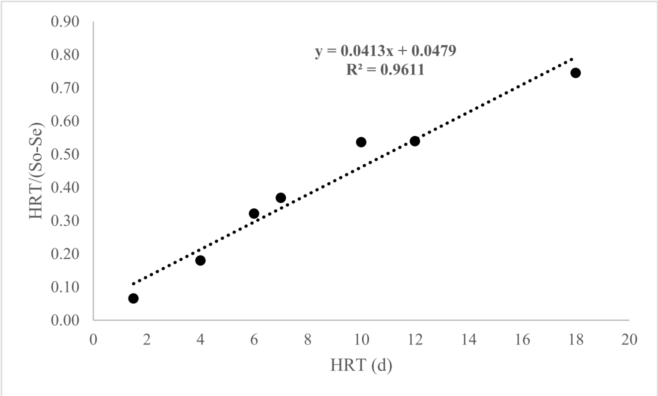

Fig. 3. Data application to the modified Stover-Kincannon model.

3.3. BMP tests

The effluent of the fermentation tests underwent to BMP trials for the definition of the residual biomethanation potential of the fermented material. In particular, the aim was to evaluate the fermentation output application in a hypothetical methanogenesis phase. In detail, output of HRT 4.0 d, 6.0 d, 7.0 d, 10.0 d, 12.0 d were considered, and as reported in Materials and Methods paragraph, also a fresh SMB (HRT 0.0 d) was tested to compare the hydrolytic effect of the fermentation phases. The results of the BMP analyses are reported below in Table 5 and Fig. 4.

As shown above, all the tests managed to produce methane with yields greater than 100 LCH_4/KgVS, consequently, this proves the feasibility of the methane production from a second reactor located downstream of the fermentation. In addition, the digestion of outputs HRT 4.0 d, 6.0 d and 7.0 d, reported better methane yield compared to the fresh substrate, emphasising that the feedstock has undergone to the breakdown of the complex lignocellulosic matrix due to the fermentation process (Sukphun et al., 2023). The separation of dark fermentation

Table 5
Total biogas and methane yields resulted from the BMP tests.

HRT	Total	CH_4
d	L/KgVS	LCH_4/KgVS
0.0	232.73	161.05
4.0	280.44	188.18
6.0	281.44	204.61
7.0	269.44	181.33
10.0	239.92	155.30
12.0	257.91	120.30

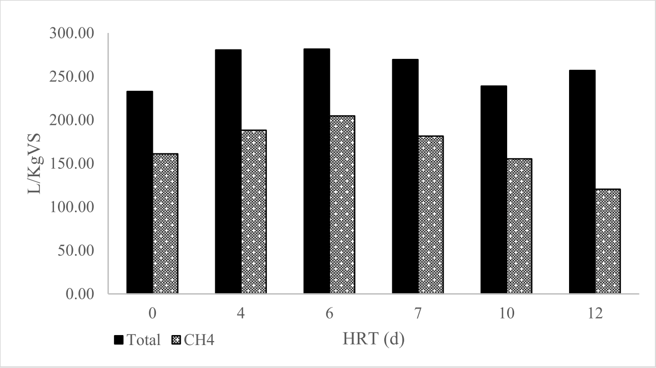

Fig. 4. Graph of the yield trends of biogas and methane produced from fermented output originated at different HRTs.

in a dedicated chamber with different operation parameters (as pH values and HRTs) avoided microbial competition, causing an improvement of the hydrolysis of the complex organic matter compared to a classic mono stage AD (Mozhiarasi et al., 2023). Consequently, it resulted an enhancement of the substrate's bioavailability in favor of methanogens behaviour. As reported from the results, the best biogas and methane yields have been achieved by the output of HRT 6.0 d with 281.44 Lbiogas/KgVS and 204.61 LCH_4/KgVS, respectively. And then, the fermentation process at HRT 6.0 d managed to improve the feedstock's biomethanation potential of 27% (v/v). Propionic acid (0.71 g/L) and butyric acid (0.51 g/L) contents were lower than the methanogenesis inhibition values corresponding to 0.9 g/L and 1.8 g/L (Wang et al., 2009); consequently, they were used as substrate to produce acetic and subsequently methane. Moreover, the VFAs concentration in the fermented output is not sufficient to determine the methane yield, in fact HRT 10.0 d reported a lower yield with the highest VFAs content (3.76 gCOD/L), but the potential of the biodegradable fraction not yet converted must also be considered.

The better performance of the TSAD was also confirmed by the energetical balance. According to the methods and the equations (5) and (6), reported in the paragraph 2.6, the energy from hydrogen and methane production (HRT 6) was of 100.2 kJ, while the one from the methane production of the mono stage AD (HRT 0) was lower of about the 30%, corresponding to 76.8 kJ.

4. Conclusions

Fermentation and following BMP tests of SMB were carried out in different condition of retention time. The main outcomes of the work were:

- Fermentation trial with low HRT promoted the washout of the methanogenic microorganisms, while methane production was observed for HRT higher than 12 days.
- The highest VFAs yield was obtained at HRT 10.0 d, while the best productivity was reached by HRT 4.0 d, 82.20 ± 10.63 gCODVFAs/KgVS and 17.09 ± 0.39 gCODVFAs/(KgVS*d), respectively.
- HRT 6.0 d reported the best hydrogen productivity with 7.98 LH_2/(KgVS*d).
- *Rmax* of 20.88 gVS/(L*d) and a *Kb* of 38.46 gVS/(L*d) were found to predict the substrate removal using the modified Stover-Kincannon model, with a maximum average error of 8%.
- The TSAD favored an energy recovery higher than 30% compared to the monostage AD, through the sequential production of hydrogen and methane.

SMB substrate can be used as feedstock for hydrogen and methane production in a TSAD process.The HRT value results a key parameter for the product differentiation. Further analysis will be evaluated to verifying the application of the knowledge acquired to other agricultural residues, but that conserving the lignocellulosic nature, as for example corn residues, triticale residues and bovine manure.

CRediT authorship contribution statement

Davide Bertasini: Data curation, Writing – original draft, Writing – review & editing. **Federico Battista:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing, Funding acquisition. **Rosa Mancini:** Data curation, Formal analysis. **Nicola Frison:** Methodology, Validation. **David Bolzonella:** Funding acquisition, Supervision, Writing – original draft.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Federico Battista reports financial support was provided by National Institute for Insurance against Accidents at Work. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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