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Bulking agent in dry anaerobic digestion as a key factor for the enhancement of biogas production

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ABSTRACT

Dry anaerobic digestion (dry-AD) is an attractive process for solid wastes such as agri-food waste. However, some limitations mainly associated to lack of effective mixing, can hinder the methane production capacity of the systems. Bulking agent (BA) has been proposed as a solution to the compaction issues in systems without mechanical agitation, such as leaching bed reactors. However, effects of BA are still not clear, and, thus, the factors to consider for its dose has not been optimized yet. This work studies the effect of BA in dry-AD. Two substrates with different characteristics were proposed as models, bean peel as a lignocellulosic substrate and a mixture of food waste as a readily biodegradable substrate. Inert plastic rings were used as BA at different BA:S ratios. Assessed BA:S ratio did not affect the performance of methane production for the lignocellulosic waste, but it did significantly affect to the easily biodegradable substrate, showing up to a 28% of methane production increase. This result could be due to the presence of lignocellulosic compounds in the bean peel, behaving like a natural BA. In assays with an increased bed height, the compaction of the system was more severe, resulting in the rapid acidification of the processes. At these conditions, the positive effect of BA addition was more marked, allowing methane production and no acidification of the system. Thus, the addition of BA is a suitable strategy for improving methane production or stability in dry-AD systems without requiring the stirring of the systems.

1. Introduction

Anaerobic digestion is a well-stablished biotechnology which allows the conversion of organic substrates into biogas by the combined action of a microbial consortium [1,2]. This technology is being implemented in many countries as a feasible and sustainable management alternative to landfill disposal or incineration of organic waste as well as to overcome natural gas dependence [3,4]. In fact, according to calculations from European Biogas Association, and in accordance with the assumptions of the European Commission from 2019, biogas should replace up to 10% of the EU27 gas demand by 2030 and up to 30–40% by 2050 (EBA) [4,5]. For achieving that, the residual biomass generated by the agri-food sector poses a crucial importance, currently being the substrate used for generating around 63% of the total biogas in Europe [6].

Among the different anaerobic digestion technologies, dry anaerobic digestion (dry-AD), so-called solid-state anaerobic digestion, is an interesting operational mode for substrates with high solid content, i.e., 15–40% of total solid content [2,7]. The possibility of operating the anaerobic digesters at this total solid range makes it attractive for biomethanization; particularly attractive for the treatment of the organic fraction of municipal solid waste or agri-food waste [8,9]. Besides that,

Abbreviations: ADR, Anaerobic digestion reactors; BA, Bulking agent; COD, Chemical oxygen demand; Dry-AD, Dry anaerobic digestion; *G*, Cumulative specific methane production; G_{max}, Ultimate methane yield coefficient; IA/PA ratio, Intermediate alkalinity/partial alkalinity ratio; LBR, Leaching bed reactor; N-NH_4^+, ammonium; OLR, Organic loading rate; R_{max}, Maximum methane production rate; S, Substrate; *t*, Time; TF, Transference Function; TS, Total solid; VFA, Volatile fatty acids; VS, volatile solid; λ, Lag time.

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dry-AD also presents some other advantages with respect to wet anaerobic digestion (total solid content below 10%), such as smaller reactor volumes, lower energy requirements for heating and/or mixing, as well as a lower moisture content in the final digestate that facilitates its handling [2,10]. Moreover, other reported advantages for dry-AD include a reduced need of water addition and a higher organic loading rate (OLR) potential than conventional wet anaerobic digestion [9,11].

Despite the many advantages, dry-AD still has some limitations that have hindered its widespread. Some of the main drawbacks are the lower biogas productivity and the longer time required for substrate degradation, in comparison to the wet anaerobic digestion [12,13]. Likewise, the operation at high total solid content would entail a potential accumulation of compounds at concentrations above the inhibitory thresholds, e.g., ammonia or volatile fatty acids (VFA), affecting the methane production [2,9,14]. In this line, the high total solid content hinders the mixing and homogenizing in dry-AD system and, thus, makes difficult the mass transfer of substrates and/or intermediate metabolites in the digesters [2]. Previous research reported that the mass diffusion coefficients in dry-AD are up to two orders of magnitude below the normal range in wet anaerobic digestion [15,16]. This difference can be explained due to the lack of effective mixing systems in dry-AD, preventing the mass transfer by convection and, thus, limiting it to diffusion processes [16].

In the specific case of food waste, mass transfer problems can be more challenging due to their nature. Firstly, food waste contains a high amount of readily biodegradable organic matter that can rapidly solubilised and transformed into VFA. The rapid accumulation of VFA may cause a pH drop and, thus, the inhibition of the methanogenic activity [17]. Moreover, food waste has a low macro-porosity due to a lack of structure that can derived in the compaction of the organic matter inside the digesters [18]. That compaction would entail a reduction in the homogenization capacity of the digesters and even affect the microbial growth [9,19]. As a possible solution for these drawbacks, the application of a bulking agent (BA) mixed with the substrate could diminish the compaction and, thus, favour the mass transfer in the digesters [9]. BA application also has the advantage of reducing the energy required since mechanical agitation in the digester would not be required. Although the use of BA is a standard strategy in composting for enhancing the oxygen diffusion, mass transfer and microbiota development in the compost piles [20,21]. The use of BA in dry-AD has been slightly reported, according to a recent review by Rocamora et al. [9]. Indeed, the BA has not been proved directly in anaerobic digestion reactors (ADR), but it has been evaluated in leaching bed reactor (LBR) coupled with ADR. For example, Demirer and Chen [22] reported the use of wood powder and wood chips as BA in a LBR coupled with ADR for dairy manure. These authors stated that the higher size of the wood chips with respect to wood powder resulted in a much more efficient leachability. Xu, Lam, Karthikeyan and Wong [23] evaluated the efficacies of five different BA, i.e. plastic full particles, plastic hollow sphere, bottom ash, wood chip and sawdust, for enhancing a LBR coupled with ADR of food waste. These authors did not report significant differences in the measured physicochemical parameters, such as pH, cumulative VFA or chemical oxygen demand (COD), in relation to the selected BA, but it is important to note that the LBR process was not used to produce biogas, so acidification of the process was desired. Both Demirer and Chen [22] and Xu, Lam, Karthikeyan and Wong [23] highlighted the importance of optimizing the BA to substrate ratio (BA:S ratio) to avoid additional costs and the necessity of higher reactor volumes. Despite the reported information, there is still a lack of knowledge on how to optimize the relation between the BA and the substrate according to the characteristics of the biomass to be digested. Moreover, the addition of BA to a dry-AD reactor, instead of a LBR coupled with ADR, has not been still reported to our best knowledge; also, the cause-effect relationship between substrate and BA on acidogenic and methanogenic stages is unknown.

The goal of this research was to understand the effect of the relation between the BA to substrate ratio (BA:S) in dry-AD. For that, two agri-food substrates were used as models, bean peel as a lignocellulosic substrate and a food waste mixture as a readily biodegradable and acidifiable substrate, whereas plastic rings were used as BA. The processes were evaluated in terms of methane production, organic matter composition and stability.

Table 1
Composition of the simulated food waste substrate.

Category	Content (% by weight, wet basis)	Detailed composition (%by weight, wet basis)
Animal protein	9.4	Beef mince with 10% of fat (9.4%)
Vegetables	37.7	Cabbage (3.8%), carrot (2.6%), carrot peel (1.1%), spinach (3.8%), tomato with peel (3.8%), broccoli (3.8%), potatoes (15.3%), potato peel (3.6%)
Fruits	18.9	Orange with peel (2.8%), lemon with peel (2.8%), apple with peel (5.7%), banana (4.5%), banana peel (3.7%)
Carbohydrates	34.0	Rice (14.1%), noodles (14.2%), bread (5.7%)

Table 2
Physicochemical characterization of the used bean peel, food waste and anaerobic inoculum.

Physicochemical parameter	Bean peel	Food waste	Anaerobic inoculum
TS (mg/kg)	898.5 ± 1.0	201.3 ± 1.6	115.9 ± 0.1
VS (mg/kg)	827.9 ± 0.1	196.7 ± 1.7	73.6 ± 0.1
pH	5.29	5.59	7.22
Lignin (%)	7.4 ± 0.2	1.6 ± 0.5	-
Cellulose (%)	23.2 ± 0.5	8.8 ± 0.9	-
Hemicellulose (%)	20.4 ± 0.5	19.9 ± 2.0	-

TS: Total solid, VS: volatile solid, COD: Chemical oxygen demand.

2. Materials and methods

2.1. Substrates and inoculum

The bean peel *(Phaseolus vulgaris)* was obtained from the Vega Central Market in Santiago (Chile). The bean peel was obtained after manually separating the grain from the pods. After that, it was dried up to a moisture content of 10.15% and the particle size was homogenized by sieving to particle sizes between 2.6 and 10.0 mm.

Simulated food waste was elaborated by mixing different meat, fruits, and vegetables in accordance with previously reported mixture compositions for food waste [24–26]. The specific composition of the food waste can be found in Table 1.

The anaerobic inoculum was obtained from a full-scale anaerobic digester working in Santiago, Chile. The digester had a stable operation for more than a year treating brewing residue. The physicochemical characterisation of substrates and inoculum can be found in Table 2.

2.2. Experimental set-up

Bottles of 250 mL of total volume with inoculum, substrate and BA in different ratios, were used for a modified biomethane potential assay [27] on dry-AD condition. The BA used for the variation of experimental porosity were cylindric hollow rings. They were made of Poly-lactic acid in a 3D printer and whose dimension were 10 mm high, 10 mm external diameter and 8 mm internal diameter. PLA was used as material for the BA due to its availability and high stability during mesophilic anaerobic digestion processes [28,29]. They were dosed and mixed with substrate and inoculum when preparing each bottle. Fig. 1.

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Fig. 1. Bottles drawing during experimental assays: a) preparation (substrate, BA and inoculum), b) after manual mixing and c) start-up after air purging.

In order to control the amount of solids in the test and ensure the dry-AD condition, the inoculum was previously drained, reducing its water content. Then, for conditioning, a buffer solution of 2 g/L of $NaHCO_3$, and micro and macro nutrients were added. Thickened inoculum and substrate were dosed in an inoculum-substrate ratio of 1 (both in volatile solid content base), resulting in a non-shakeable slurry with a solid content of 12% (approximately). Then, the bottles were degassed with Nitrogen and subjected to vigorous manual shaking, starting the assay. The bottles were left standing in a thermostatic chamber that kept a constant temperature of 30 °C, until the end of the operation during 30 d or until a negligible biogas production quantification.

2.3. *Experimental design*

To study the effect of the BA on the dry-AD process, two types of experiments were carried out: 1) the effect of the BA dosage and 2) the effect of fixed bed height.

2.3.1. *Effect of the bulking agent dosage*

Three dosage ratios of BA and substrate were evaluated: 1:2, 1:1 and 2:1; as well as a control without BA (0:1). For each condition, both biogas and concentration of intermediate metabolites were monitored over time. Biogas production was measured daily using a pressure transducer; its composition (methane, carbon dioxide and hydrogen sulphide) was measured by sampling and quantification in gas chromatography. The concentration of chemical oxygen demand (COD), ammonium (N-NH_4^+), total volatile fatty acids (VFA), pH and alkalinity) was quantified through several bottle destruction (two each time). The metabolites were recovered from a leachate prepared by solid-liquid extraction. For each experimental condition, the study began with 10 bottles and ended with 2.

2.3.2. *Effect of fixed bed height*

The objective of this test is to determine the effect of increasing the weight of the substrate bed, that is to say, the effect of compaction due to the weight of the substrate itself, and therefore it was evaluated to double the amount of digested material compared to the original test (2.3.1) using two BA dosage conditions, without addition of BA called control and a 1:1 dosage. In the same way that 2.3.1 section, biogas and concentration of intermediate metabolites were monitored over time. Biogas production through daily measurements by a pressure transducer and its composition (methane, carbon dioxide and hydrogen sulphide) by sampling and quantification in gas chromatography. The concentration of chemical oxygen demand (COD), ammonium (N-NH_4^+), total volatile fatty acids (VFA), pH and alkalinity) was quantified through several bottle destruction (two each time). The metabolites were recovered from a leachate prepared by solid-liquid extraction. For each experimental condition, the study began with 10 bottles and ended with 2.

2.4. *Analytical procedures*

Total solids (TS), volatile solids (VS) and chemical oxygen demand (COD) were measured through standard methods [30]. For metabolite quantification, sampled bottles were subjected to a solid-liquid extraction process for leachate recovery in a destructive way. The soluble fraction recovered was used for soluble physicochemical parameters characterization (chemical oxygen demand (COD_S), ammonia, partial and total alkalinity and VFA (estimation by using titration method) through standard methods [30]. Also, a mixture of VFA was individually quantified by HPLC (acetate, propionate, butyrate, lactate, saccharose and ethanol were measured) by using an Aminex HPX-87 H column with DAD and IR detectors [31].

The solid-liquid extraction process was performed directly into the digestion bottles after sampling. For this, 100 mL of distilled water was added to each bottle and mixed at 200 rpm at a temperature of 30 °C for 1 h (100 C JSR thermostatic shaker). Once the time had elapsed, the bottles were removed from the shaker, and the pH was directly measured (Ohaus brand Starter 2100 pH-meter). Subsequently, the bottle content was decanted, and the supernatant was placed in 50 mL Falcon tubes for centrifugation at 5000 rpm and 10 °C for 10 min (Boeco Germany model U-320 R centrifuge); for finally filtering the soluble fraction through a 0.45 µm pore size.

To measure the lignin, hemicellulose, and cellulose of bean peel and food waste, the dry samples were passed through a mill Restch 2000 and sieved between 0.40 and 0.25 mm (TAPPI T 257 cm-85), following the elimination of extractives compounds were carried up (TAPPI T204 cm-97). The measurement of the different fibre fraction was carried out according to the methodology described by the Technical Association of the Pulp and Paper Industry [32].

2.5. *Kinetic parameters*

The Transference Function (TF) model was applied to compare the kinetics of the methane production, fitting the obtained experimental data of accumulated methane production throughout the experimental time (Eq. 1). TF model has been previously applied for biomethanization of different agro-industrial organic waste [33,34].

$$G = G_{\max} * \left(1 - \exp\left[-\frac{R_{\max}(t-\lambda)}{G_{\max}}\right]\right) \tag{1}$$

In the TF G (mL CH_4/g VS_{added}) is the cumulative specific methane production, G_{max} (mL CH_4/g VS_{added}) is the ultimate methane yield coefficient, R_{max} is the maximum methane production rate (mL CH_4/(g VS_{added} d)), t (d) is the time and λ (d) is the lag time. Error (%) and r^2 were calculated to evaluate the goodness-of-fit and the accuracy of the results. The kinetics parameters were calculated using the software Sigma-Plot (version 10.0).

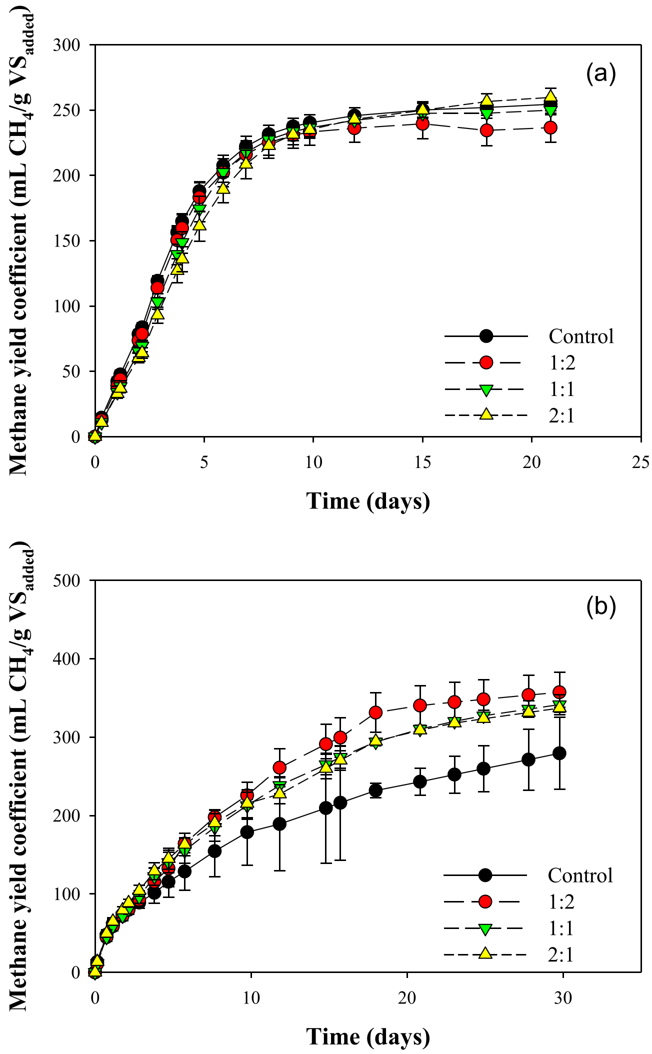

Fig. 2. Variation of the methane yield coefficient (mL CH_4/g VS_{added}) for the control (no bulking-agent) and the assessed bulking-agent to substrate ratios for a) bean peel and b) food waste, throughout the experimental time; were VS, volatile solids.

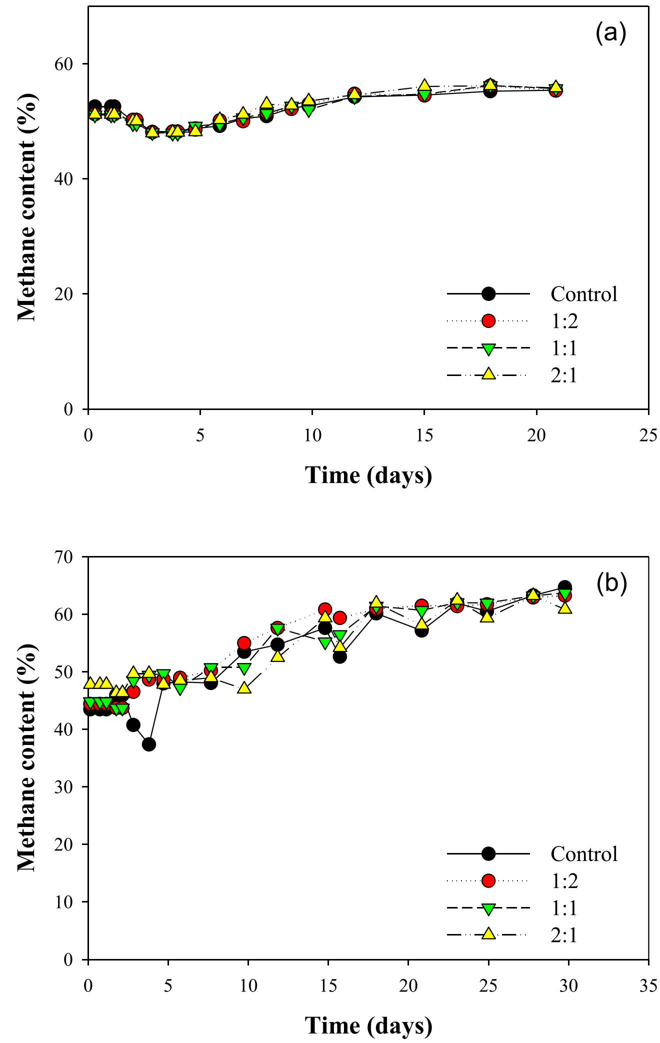

Fig. 3. Variation of the methane content (%) for the control (no bulking-agent) and the assessed bulking-agent to substrate ratios throughout the experimental time for a) bean peel and b) food waste.

3. Results and Discussion

3.1. Effect of the bulking agent dosage

3.1.1. Methane production

As shown in Fig. 2, the final methane yield coefficients of bean peel showed almost no differences in function of the BA dosage, achieving a final average value of 250 ± 9 mL CH_4/g VS_{added} (Fig. 2a). On the opposite, the addition of a BA in the dry-AD of food waste resulted in an enhancement of the final methane yield coefficient with respect to the control. Concretely, the highest final methane yield coefficient was achieved for the BA:S ratio of 1:2, i.e., 357 ± 25 mL CH_4/g VS_{added}, which entailed an increment of 28% with respect to the control (Fig. 2b). On the other hand, the addition of BA showed a poor influence on the methane content of the generated biogas for both bean peel and food waste (Fig. 3). However, the dry-AD of each substrate resulted in a different trend of the methane content. As can be seen in Fig. 3a, the methane content for bean peel shortly varied in a range of around 48% to 56%, showing all the tested conditions an average final value of 55.6 ± 0.2% at the end of the experimental time. On the contrary, the methane content for food waste increased from values around 43% up to values close to 65% at the end of the experimental time (Fig. 3b). The initially observed result seems to be contradictory due to the different nature of both residues. It is necessary to emphasize that bean peel is a lignocellulosic residue; therefore, it has more difficulty in its

Table 3
Kinetics parameters obtained from the transference function equation applied to each dry anaerobic digestion batch test.

	G_{max} (mL CH_4/g VS_{added})	R_{max} (mL CH_4/(g VS_{added}·d))	λ (d)	R^2	Error (%)
Bean peel					
Control	262 ± 5	65 ± 3	0.2 ± 0.1	0.990	2.9
1:2	249 ± 6	65 ± 4	0.2 ± 0.1	0.845	5.0
1:1	262 ± 7	59 ± 4	0.3 ± 0.1	0.984	4.6
2:1	273 ± 8	52 ± 3	0.3 ± 0.1	0.988	4.9
Food Waste					
Control	270 ± 10	32 ± 3	0	0.976	-3.5
1:2	383 ± 8	38 ± 1	0	0.996	6.7
1:1	350 ± 8	36 ± 2	0	0.993	2.4
2:1	338 ± 10	38 ± 2	0	0.986	0.3

G_{max}, ultimate methane yield coefficient; R_{max}, maximum methane production rate; λ, lag time; VS, total volatile solids.

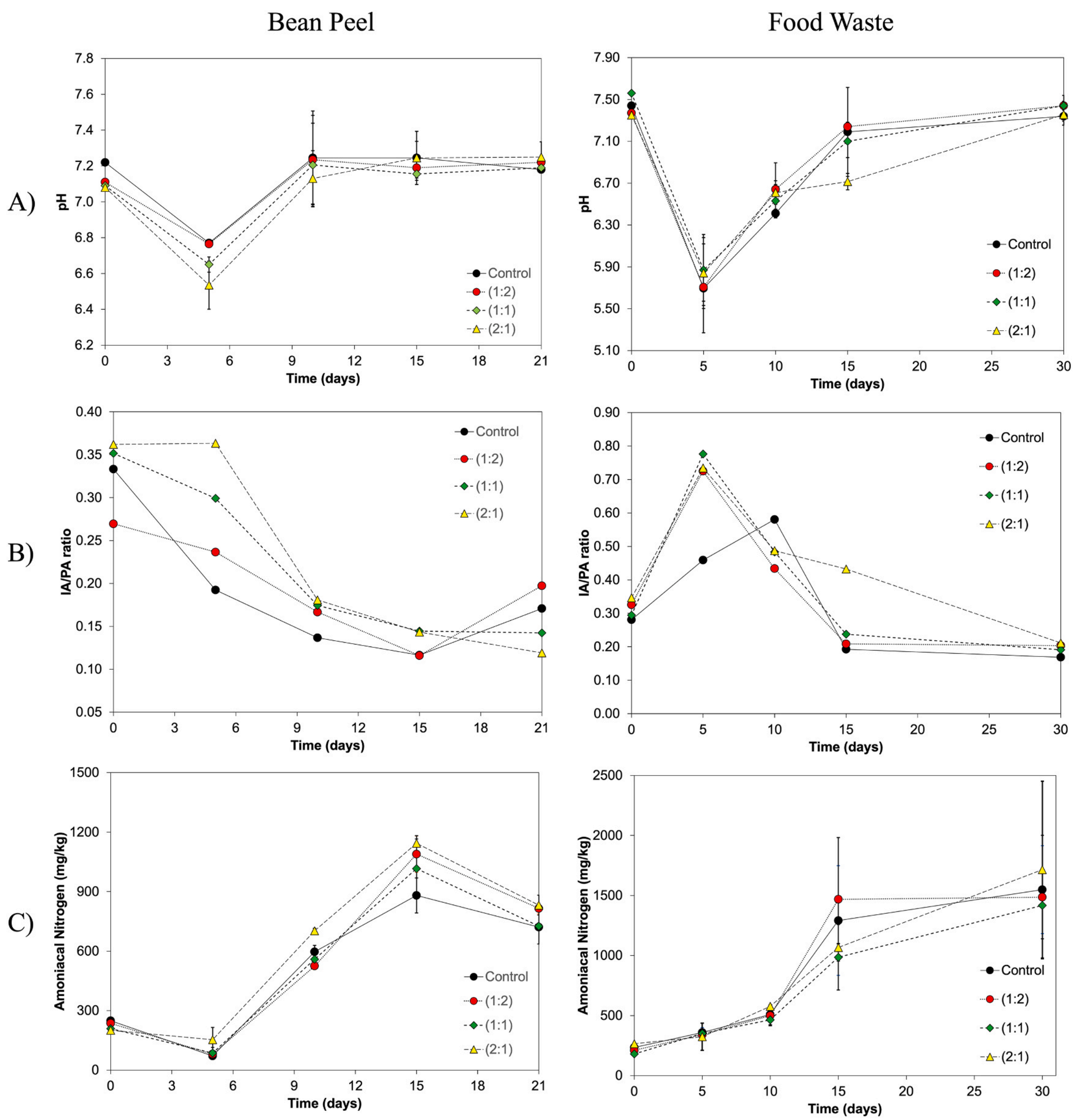

Fig. 4. Variation of the concentration of A) pH, B) intermediate alkaline to partial alkaline (IA/PA) ratio and C) the ammoniacal nitrogen for the control (no bulking-agent) and the assessed bulking-agent to substrate ratios throughout the experimental time for bean peel and food waste. The figures show the standard deviation of a duplicate.

biodegradation, and it should have lower biogas production and methane content than food waste. On the other hand, the food waste that contains almost no lignocellulosic matter and is highly biodegradable should have been easily digested. This margin between the expected result and what was observed is attributed to the physical conformation of the bed during Dry-AD, that is to say to the porosity of the digestion bed, which is responsible of the transport of gas and inhibitory metabolites. Then, the enhancement in the methane yield coefficient and methane content observed at adding BA in the digesters fed with food waste may mean that this substrate has a low porosity derived from a low content of lignocellulosic fibres (Table 2) and, thus, causing lack of bulking. Therefore, it tends to compact, hindering the transfer of matter and, thus, affecting the entire anaerobic degradation pathways [9,22]. In the opposite, the small impact of the BA addition observed for bean peel would be explained because of the high lignocellulosic content, that can provide structural support (Table 2), preventing an excessive compaction of the feedstock [35].

In order to facilitate the comparative evaluation of the different BA:S ratios, the kinetics parameters from the transference function equation (Eq. 1) are shown in Table 3. As can be seen, the values obtained for G_{max} were in line with the experimental values shown in Fig. 2. In fact, the errors, calculated as the percentual difference between the experimental methane yield coefficient and G_{max}, were lower than 5% in most of the cases, showing the good fitting of the applied model (Table 3). The variation of the BA:S ratio resulted in differences in the maximum methane production rates for both assessed substrates. For bean peel, the maximum methane production rate was very similar for the control and for BA:S ratios of 1:2 and 1:1, reaching an average value of 63 ± 3 mL CH_4/(g VS_{added}·d). However, the increment of the BA in the ratio 2:1 resulted in a decrease of the maximum methane production rate of around 20% with respect to the control (Table 3). The lack of enhancement by adding a BA could be explained by the lignocellulosic content of the bean peel, i.e., 7.4 ± 0.2% lignin and 23.2 ± 0.5% cellulose (Table 2), which would be enough to avoid the compaction of the digesters bed and allow the mass transfer [35]. However, an excess of BA in the BA:S ratio of 2:1 would have affected the kinetics of the anaerobic digestion by heat losses or by hindering contact between microorganisms and the substrate [36]. On the contrary, the addition of BA to the

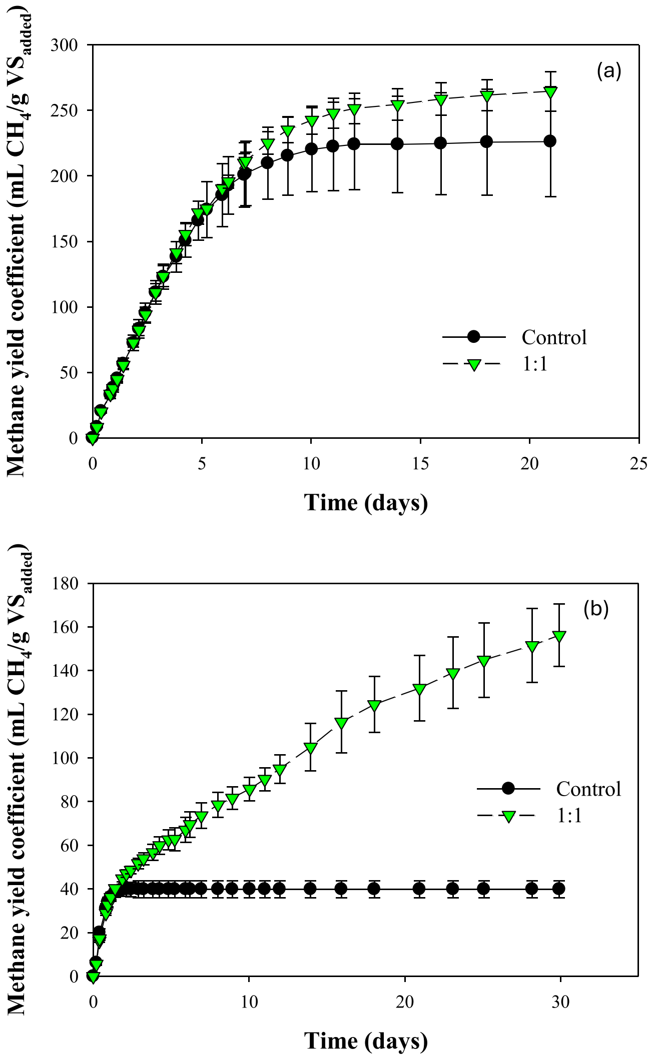

Fig. 5. Variation of the methane yield coefficient (mL CH_4/g VS_{added}) for the control (no BA) and at BA/S ratio of 1:1 for a) bean peel and b) food waste, throughout the experimental time; were VS, volatile solids.

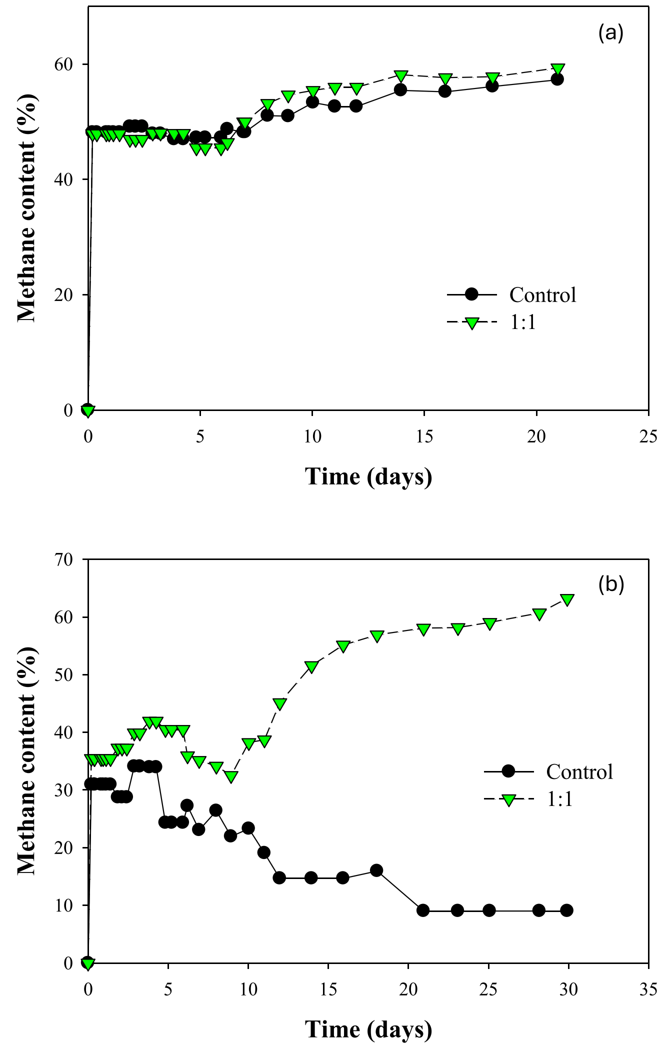

Fig. 6. Variation of the methane content (%) with and without Bulking agent throughout the experimental time for a) bean peel and b) food waste.

dry-AD of food waste enhanced the maximum methane production rate by around 15% with respect to the control (Table 3). That would be explained by the enhancement of the mass transfer in the digesters by adding the BA, improving the microbial access to the substrate and intermediates [9]. No significant differences in the maximum methane production rate were determined among the different assessed BA:S ratios, obtaining an average value of 37 ± 1 mL CH_4/(g VS_{added}·d).

3.1.2. Stability and soluble compounds

Three parameters were monitored for evaluation in stability of the reactors for both substrates used due to BA dose: pH, IA/PA ratio and Ammoniacal Nitrogen (Fig. 4). In the case of pH (Fig. 4. A), the different BA:S ratios showed little impact on the evolution of the pH. For bean peel, the pH decreased significantly on the fifth day of digestion, reaching a value of 6.5–6.8. Then, it increased to recover a value close to neutrality and remained constant until the end of the test. In the case of food waste, the pH also decreased significantly on the fifth day to a value of 5.7–5.9, but the increase to neutrality occurred slowly.

In the case of the IA/PA ratio (Fig. 4B), different behaviour was also observed for each residue. The bean peel showed a tendency to decrease constantly from values close to 0.3 to a final ratio of 0.15. Regarding the effect of BA, it could be indicated that the lower the BA content, the faster the alkalinity ratio decreased, which was manifested with greater significance on the fifth day. In the case of food residue, the trials with BA had different behaviours than the control trial, where the alkalinity ratio reached a maximum on the fifth day of digestion with an approximately value of 0.75, and then gradually decreased until reaching a minimum of 0.2. On the other hand, the control trial showed a gradual increase in the ratio, reaching a maximum on the tenth day of digestion and dropping abruptly. This behaviour could indicate an acceleration of the digestion process due to BA on food waste but not on the bean peel. In all cases, it can be indicated that the dry-AD processes were successful because they were capable of remaining at values lower than 0.4 throughout the experimental time, indicating a stable performance of the dry-AD reactor operation [37].

The different BA:S did not significantly impact the concentration of ammoniacal nitrogen of the dry-AD reactor fed with bean peel or food waste (Fig. 4C). For both substrates, the concentration of ammoniacal nitrogen increased during operation time, reaching average values at the end of the assay of 773.53 ± 58 mg/L and 1541 ± 127 mg/L for bean peel and food waste, respectively (Fig. 4C). These values are at the lower limit of the concentrations reported as inhibitory for the dry-AD process, whose inhibition threshold has been defined at values higher than 1500 to 4000 mg/L [9,38].

Finally, it is possible to indicate that all the experimental conditions studied did not present large deviations instability due to the dosage of BA, but that due to the nature of the residues (lignocellulose versus

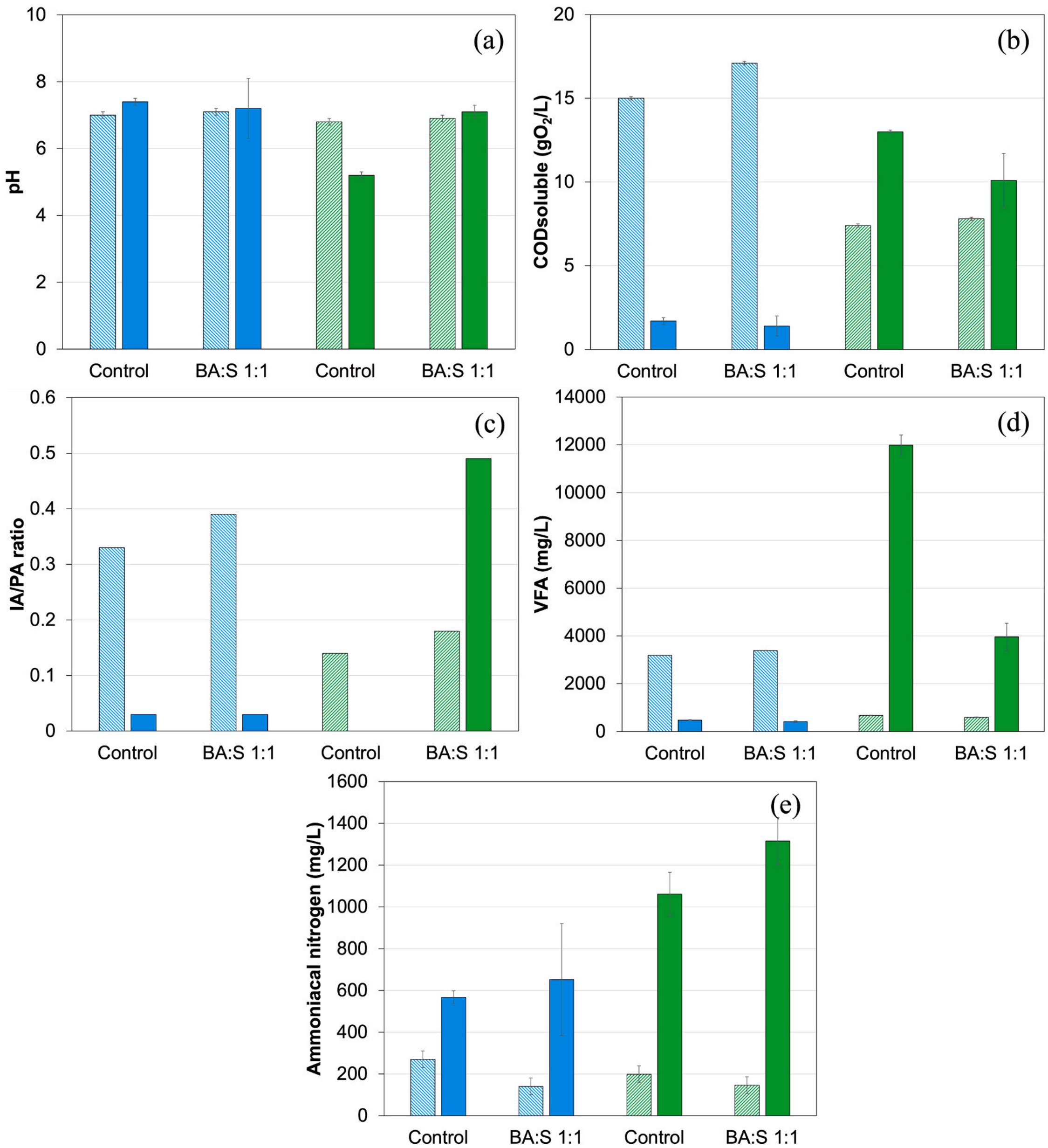

Fig. 7. Variation of the concentration of a) pH, b) soluble chemical oxygen demand (CODsoluble), c) intermediate alkaline to partial alkaline (IA/PA) ratio, d) volatile fatty acids (VFA) concentration, and e) ammoniacal nitrogen of the digesters fed with bean peel and food waste at the initial and final time of the experiments. The figures show the standard deviation of a duplicate. (Legend: light blue colour is bean peel and green colour is food waste. Bars with lines corresponds to initial time and the solid bars are final time).

easily biodegradable), different behaviours were observed as a function of time for the monitored metabolites.

3.2. *Effect of fixed bed height*

3.2.1. *Methane production*

Increasing the height of biomass in the reactors resulted in a significant effect of the BA addition to the digesters on the methane yield coefficient and methane content in both studied substrates (Figs. 5 and 6). Unlike previously observed (Fig. 2), the addition of BA to the digesters fed with bean peel resulted in an enhancement of the methane yield coefficient, which increased up to 17% with respect to the control (Fig. 5a).

This positive effect was much more marked in the digesters fed with food waste (Fig. 5b), where the methane production increased from 40 ± 9 mL CH_4/g VS_{added} to 156 ± 63 mL CH_4/g VS_{added}, i.e., 292% higher, by adding BA respect to digester without BA. That would be explained by an increase in the compaction of the digester bed due to the increase in the bed height with respect to the results discussed in Section 3.1. Besides, the higher impact of the addition of BA in the digesters fed with food waste with respect to the digesters fed with bean peal could be expected due to the lower content in lignocellulosic fibres of the food waste (Table 2), which facilitated the compaction of the digesters and, thus, affected the homogenization capacity and even the microbial growth [9,19]. With respect to the methane content (Fig. 6), it does not show a marked difference in the digesters fed with bean peel despite the added BA, reaching values close to 60% at the end of the experimental time at both conditions (Fig. 6a). On the contrary, the highest impact of the BA addition on methane content was observed for the digester fed with food waste, where the operation without BA resulted in the decrease of the methane content in the biogas throughout the experimental time up to values below 10% (Fig. 6b). On the contrary, the

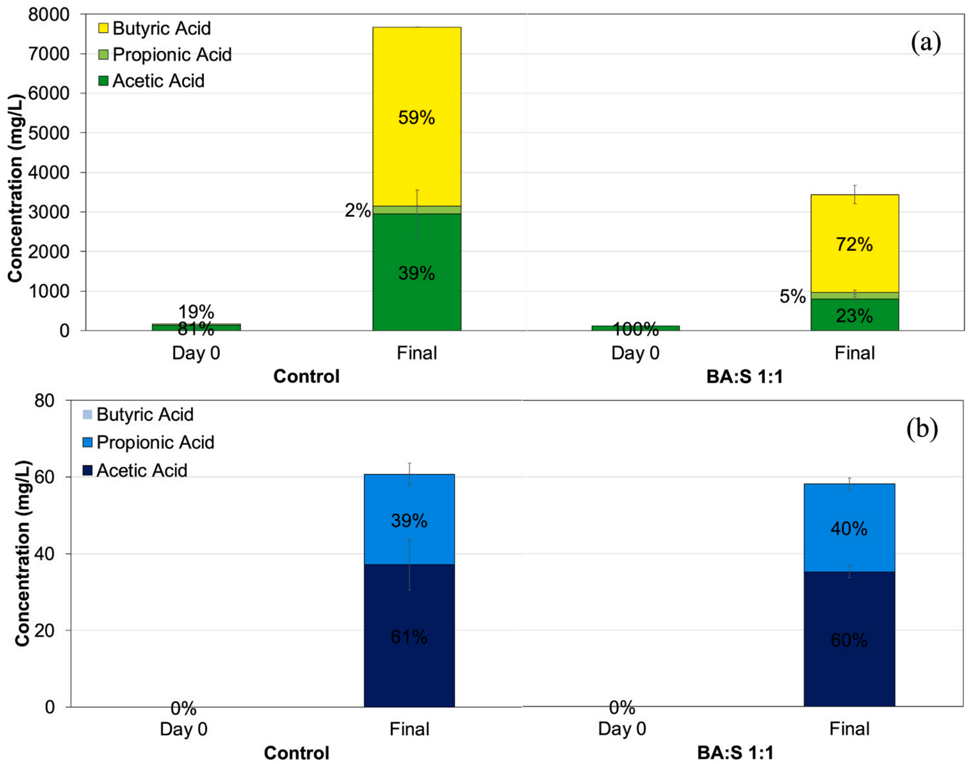

Fig. 8. Quantification of soluble organic compounds by HPLC, which only detected acetic, butyric and propionic acids, under the different conditions studied: a) Food waste and b) Bean peel. The figures show the standard deviation of a duplicate.

dry-AD reactor with BA fed with food waste showed a methane content of around 60% after 20 days of operation. The kinetics parameter showed a trend in line with the differences observed for the methane yield coefficient (Supplementary material, Table S1).

3.2.2. Stability and soluble compounds

For dry-AD fed with been peel, despite of the enhancement observed in the methane yield coefficient (Fig. 5a), the addition of BA did not entail remarkable differences in the physical-chemical characterisation of the digesters at the end of the experimental time. On the contrary, for dry-AD fed with food waste, the improvement by the addition of BA was also observed in the physical-chemical characterisation of the digesters (Fig. 7). Concretely, the dry-AD operation with and without BA addition using bean peel as substrate allowed a pH within this optimal range at the end of the experimental time (Fig. 7a). As can be seen in Fig. 7b, the operation of digesters fed with food waste without BA resulted in the acidification of the process, with a pH of 5.2 ± 0.1 at the end of the experimental time. In fact, the quantification of these effects showed a significant variation for the food waste without BA (control), being statistically lower than the initial value (P(t)= 0.0162), and also, it was lower than the pH obtained when the BA was applied (P(t)= 0.000212). These results indicate that the presence of the BA effectively reduces the pH drops. In addition, this value would reinforce the inhibitory effect of biogas production, over the inoculum pretreatment, and explain the low methane yield coefficient observed at this condition, since the methanogenic activity is drastically inhibited at a pH outside the range 6.6–7.8 [39,40]. Nevertheless, the use of a BA favoured the dry-AD, allowing a pH within the optimal range for the methanogenic activity [39,40].

With respect to COD soluble (Fig. 7b). In reactors for bean peel with and without BA was possible the reduction of the soluble COD around 90% respect to the initial concentration, while using food waste as substrate it was not possible to reduce the COD, obtaining an increase. Also, the data shows an apparent reduction in the soluble COD caused by the BA for both residues; however, upon quantifying the effect, it was determined that the differences were negligible. The consumption of soluble organic matter in a reactor with and without BA using bean peel as substrate was in line with the drop of the IA/PA ratio to 0.03 at the end of the experimental time (Fig. 7c), showing a very stable operation [37]. Instead, for food waste as substrate in reactor with BA the IA/PA was close to 0.5.

The digestors with BA resulted in a slight increase of the ammoniacal nitrogen concentration respect to digestors without BA for both substrates (Fig. 7e); which, despite having the same behaviour in both residues, resulted to be negligible statistically. That would indicate an easier access to the microorganisms to the substrates or that this specific condition facilitated the metabolism of the nitrogenous compounds (proteins) present in each residue; resulting in a higher biodegradation with a higher release of ammoniacal nitrogen. Besides, the determined concentrations of ammoniacal nitrogen in all the cases were lower than the concentrations reported as inhibitory for the dry anaerobic digestion process, i.e., 1500 to 4000 mg/L [9,38].

The VFA content (Figs. 7d and 8b) from bean peel, decreased significantly around 85% (Fig. 7d) for the digester with and without BA (P(t)= 0.00138 for control and P(t)= 0.00132 for BA 1:1), showing a stable operation for this condition and a very small residual amount, mainly composed by acetic acid (Fig. 8b). On the contrary, for the digesters with and without BA, the ease of generating VFA from food waste [17] resulted in non-optimal stability values increasing significantly the total VFA amount (P(t)= 0.000849 for control and P(t)= 0.0185 for BA 1:1). Indeed, the increment of VFA (Fig. 7d) was in line with the pH at the end of the experimental time, showing a higher significant acidification of the dry-AD process without BA (Fig. 7a) being the concentration reached by the control three times that of the system with BA (P (t)= 0.00435). In this case, the dominant compound was the butyric acid over the acetic acid (Fig. 8b). Generally, it is known that the imbalance in the acid content is a sign of inhibition of anaerobic digestion, which has also been observed in the specific case of butyric acid in dry anaerobic digestion process [41]. On the other hand, the BA in the digesters fed with food waste favoured the consumption of VFA, which presented a final value of 3958 ± 570 mg/L, i.e., 67% less than without BA, although still presenting a high proportion of butyric acid over acetic acid (Fig. 8a). This difference in the VFA concentration for the digesters fed with food waste was in line with the observed differences in methane production (Fig. 5b), indicating that the addition of BA favoured the methanogenic activity, probably due to the enhancement of the mass transfer that could facilitate to the methanogens the access to the VFA [9]. Despite of the improvement in the VFA consumption, for food waste as fed to digesters with BA still showed a partial accumulation of soluble COD, probably indicating that the microorganisms did not exhaust the consumption of organic matter at the end of the experimental time. This incomplete biodegradation would also explain the determined IA/PA ratio (Fig. 7c), slightly above the limit for a stable operation [37].

Thus, as it was concluded from the evaluation of the methane production, the low lignocellulosic content of food waste (Table 2) would favour the compaction of the reactor, and, therefore, the addition of a BA favoured the dry-AD reactor. Contrary, the bean peel has enough lignocellulosic content so as not to require a BA that improves mass transfer.

4. Conclusions

The addition of BA is a suitable strategy for improving the methane production and stability in dry-AD systems. The positive effect of the BA can be related to an enhancement of the porosity in the digester bed, which reduces the compaction and, thus, favours the mass transfer during the dry-AD process. In that sense, the optimal BA:S ratio was dependent on the characteristics of the substrate, being observed a higher enhancement of the parameters for food waste, a substrate with lower lignocellulosic content and more acidifiable than bean peel. The positive effects associated with the addition of BA were also higher at increasing the bed height due to the higher impact of the compaction effects in the digester beds.

CRediT authorship contribution statement

Andrea Carvajal: Writing – review & editing, Supervision, Project administration, Conceptualization. **Ignacio Poblete-Castro:** Writing – review & editing, Supervision, Project administration. **Fernanda Pinto-Ibieta:** Writing – review & editing, Methodology. **Claudio Sepúlveda:** Writing – original draft, Methodology. **Daniel Navia:** Writing – review & editing, Supervision. **Antonio Serrano:** Writing – original draft, Formal analysis.

Declaration of Competing Interest

The authors 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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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.nbt.2024.05.002.

References

[1] Wheatley A. Amsterdam. Anaerobic digestion: a waste treatment technology. Springer Netherlands; 1990.
[2] Wang Z, Hu Y, Wang S, Wu G, Zhan X. A critical review on dry anaerobic digestion of organic waste: characteristics, operational conditions, and improvement strategies. Renew Sust Energ Rev 2023;176:113208.
[3] Serrano A, Villa-Gomez D, Fermoso FG, Alonso-Fariñas B. Is anaerobic digestion a feasible alternative to the combustion of olive mill solid waste in terms of energy production? A critical review. Biofuel Bioprod Biorefin 2021;15(1):150–62.
[4] Sulewski P, Ignaciuk W, Szymańska M, Wąs A. Development of the Biomethane Market in Europe. Energies 2023;16(4).
[5] European Commission. Clean energy for all Europeans. Directorate-General for Energy; 2019.
[6] Calderón C., Geelen J., Jossart J.M., Decorte M. Bioenergy Europe's 2022 Report on Biogas, 2022.
[7] Cho SK, Im WT, Kim DH, Kim MH, Shin HS, Oh SE. Dry anaerobic digestion of food waste under mesophilic conditions: performance and methanogenic community analysis. Bioresour Technol 2013;131:210–7.
[8] Guendouz J, Buffière P, Cacho J, Carrère M, Delgenes JP. Dry anaerobic digestion in batch mode: design and operation of a laboratory-scale, completely mixed reactor. Waste Manag 2010;30(10):1768–71.
[9] Rocamora I, Wagland ST, Villa R, Simpson EW, Fernández O, Bajón-Fernández Y. Dry anaerobic digestion of organic waste: a review of operational parameters and their impact on process performance. Bioresour Technol 2020;299:122681.
[10] Wang Z, Jiang Y, Wang S, Zhang Y, Hu Y, Hu ZH, Wu G, Zhan X. Impact of total solids content on anaerobic co-digestion of pig manure and food waste: insights into shifting of the methanogenic pathway. Waste Manag 2020;114:96–106.
[11] Karthikeyan OP, Visvanathan C. Bio-energy recovery from high-solid organic substrates by dry anaerobic bio-conversion processes: a review. Rev Environ Sci Biotechnol 2013;12(3):257–84.
[12] Angelonidi E, Smith SR. A comparison of wet and dry anaerobic digestion processes for the treatment of municipal solid waste and food waste. Water Environ J 2015; 29(4):549–57.
[13] Hu Yy, Wu J, Li HZ, Poncin S, Wang KJ, Zuo JE. Study of an enhanced dry anaerobic digestion of swine manure: performance and microbial community property. Bioresour Technol 2019;282:353–60.
[14] Sun C, Cao W, Banks CJ, Heaven S, Liu R. Biogas production from undiluted chicken manure and maize silage: a study of ammonia inhibition in high solids anaerobic digestion. Bioresour Technol 2016;218:1215–23.
[15] Xu F, Wang ZW, Tang L, Li Y. A mass diffusion-based interpretation of the effect of total solids content on solid-state anaerobic digestion of cellulosic biomass. Bioresour Technol 2014;167:178–85.
[16] Bollon J, Benbelkacem H, Gourdon R, Buffière P. Measurement of diffusion coefficients in dry anaerobic digestion media. Chem Eng Sci 2013;89:115–9.
[17] Li Y, Park SY, Zhu J. Solid-state anaerobic digestion for methane production from organic waste. Renew Sust Energ Rev 2011;15(1):821–6.
[18] Ameen HA, Dohuki MSSM. Effect of leached and co-composted organic fraction of municipal solid waste with bulking agents on soil properties and sweet pepper plant development under calcareous soil. Commun Soil Sci Plant Anal 2023;54 (12):1627–43.
[19] Buffière P, Steyer JP, Fonade C, Moletta R. Modeling and experiments on the influence of biofilm size and mass transfer in a fluidized bed reactor for anaerobic digestion. Water Res 1998;32(3):657–68.
[20] Manish B, Richa G, Archana TJJoB. Implementation of bulking agents in composting: a review. Bioremed Biodegrad 2013;4:7.
[21] Ledezma-Villanueva A, Robledo-Mahón T, Gómez-Silván C, Angeles-De Paz G, Pozo C, Manzanera M, Calvo C, Aranda E. High-Throughput Microbial Community Analyses to Establish a Natural Fungal and Bacterial Consortium from Sewage Sludge Enriched with Three Pharmaceutical Compounds. J Fungi 2022;8(7):668.
[22] Demirer GN, Chen S. Anaerobic biogasification of undiluted dairy manure in leaching bed reactors. Waste Manag 2008;28(1):112–9.
[23] Xu SY, Lam HP, Karthikeyan OP, Wong JWC. Optimization of food waste hydrolysis in leach bed coupled with methanogenic reactor: Effect of pH and bulking agent. Bioresour Technol 2011;102(4):3702–8.
[24] Bouallagui H, Lahdheb H, Ben Romdan E, Rachdi B, Hamdi M. Improvement of fruit and vegetable waste anaerobic digestion performance and stability with co-substrates addition. J Environ Manag 2009;90(5):1844–9.
[25] Komemoto K, Lim YG, Nagao N, Onoue Y, Niwa C, Toda T. Effect of temperature on VFA's and biogas production in anaerobic solubilization of food waste. Waste Manag 2009;29(12):2950–5.
[26] Yin J, Yu X, Zhang Y, Shen D, Wang M, Long Y, Chen T. Enhancement of acidogenic fermentation for volatile fatty acid production from food waste: Effect of redox potential and inoculum. Bioresour Technol 2016;216:996–1003.
[27] Angelidaki I, Alves M, Bolzonella D, Borzacconi L, Campos JL, Guwy AJ, Kalyuzhnyi S, Jenicek P, van Lier JB. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. Water Sci Technol 2009;59(5):927–34.

[28] Yagi H, Ninomiya F, Funabashi M, Kunioka M. Anaerobic biodegradation tests of poly(lactic acid) and polycaprolactone using new evaluation system for methane fermentation in anaerobic sludge. Polym Degrad Stab 2009;94(9):1397–404.
[29] Álvarez-Méndez SJ, Ramos-Suárez JL, Ritter A, Mata González J, Camacho Pérez A. Anaerobic digestion of commercial PLA and PBAT biodegradable plastic bags: potential biogas production and ^{1}H NMR and ATR-FTIR assessed biodegradation. Heliyon 2023;9(6):e16691.
[30] A. American Public Health, A.D. Eaton, A. American Water Works, F. Water Environment, Standard methods for the examination of water and wastewater, APHA-AWWA-WEF, Washington, D.C., 2005.
[31] de Sá LRV, de Oliveira MAL, Cammarota MC, Matos A, Ferreira-Leitão VS. Simultaneous analysis of carbohydrates and volatile fatty acids by HPLC for monitoring fermentative biohydrogen production. Int J Hydrog Energy 2011;36 (23):15177–86.
[32] Tappi Test Methodos Standard Methods for Pulp and Paper, Tappi Press, Atlanta, GA, USA, 1997.
[33] Donoso-Bravo A, Pérez-Elvira SI, Fdz-Polanco F. Application of simplified models for anaerobic biodegradability tests. Evaluation of pre-treatment processes. Chem Eng J 2010;160(2):607–14.
[34] Serrano A, Fermoso FG, Rodríguez-Gutierrez G, Fernandez-Bolaños J, Borja R. Biomethanization of olive mill solid waste after phenols recovery through low-temperature thermal pre-treatment. Waste Manag 2017;61:229–35.
[35] Kulikowska D, Bernat K, Zaborowska M, Zielińska M. Municipal sewage sludge composting in the two-stage system: the role of different bulking agents and amendments. Energies 2022;15(14):5014.
[36] Kulcu R, Yaldiz O. Composting of goat manure and wheat straw using pine cones as a bulking agent. Bioresour Technol 2007;98(14):2700–4.
[37] Martín-González L, Font X, Vicent T. Alkalinity ratios to identify process imbalances in anaerobic digesters treating source-sorted organic fraction of municipal wastes. Biochem Eng J 2013;76:1–5.
[38] Chen Y, Cheng JJ, Creamer KS. Inhibition of anaerobic digestion process: a review. Bioresour Technol 2008;99(10):4044–64.
[39] Lay JJ, Li YY, Noike T. Influences of pH and moisture content on the methane production in high-solids sludge digestion. Water Res 1997;31(6):1518–24.
[40] Demirel B, Scherer P. The roles of acetotrophic and hydrogenotrophic methanogens during anaerobic conversion of biomass to methane: a review. Rev Environ Sci Biotechnol 2008;7(2):173–90.
[41] Schievano A, D'Imporzano G, Malagutti L, Fragali E, Ruboni G, Adani F. Evaluating inhibition conditions in high-solids anaerobic digestion of organic fraction of municipal solid waste. Bioresour Technol 2010;101(14):5728–32.