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Research Paper

The fate of biodegradable plastic during the anaerobic co-digestion of excess sludge and organic fraction of municipal solid waste

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ABSTRACT

Co-digestion of the organic fraction of municipal solid waste (OFMSW) and excess sludge has several benefits especially related to improved methane production and better process stability. In recent years, the presence of biodegradable plastics is increasingly common in OFMSW especially since, as in Italy, biodegradable bags are used for its collection. In this paper, the influence and the fate of biodegradable bags during anaerobic co-digestion of excess sludge and OFMSW are assessed. The best results in terms of methane yield (about 180 NmL/g_{VS}) have been obtained with the 50:50 (VS basis) co-digestion of excess sludge and OFMSW with an organic loading rate of 2 $kg_{VS}/m^3 \cdot d$. Bioplastic degradation is very limited during the co-digestion but it does not influence methane production or digestate chemical characteristics. However, the feeding of bioplastic bags seems to induce a higher phytotoxicity and the presence of undigested fragments is anyway a problem for further treatment or direct utilization of digestate.

1. Introduction

Food waste collection is one of the main applications for bioplastic materials and biodegradable/compostable bags are considered a preferential option for the collection of the organic fraction of municipal solid waste (OFMSW) (Folino et al., 2023). According to Italian legislation, biodegradable bags have to fulfill the technical standards UNI EN 13432:2002 for packaging and UNI EN 14995:2007 for other materials to be accepted in biological treatment plants. Their use would allow to avoid the preliminary separation from OFMSW thus simplifying waste management (Abraham et al., 2021). However, since bioplastics cannot be differentiated by visual inspection from conventional plastics, they are often removed before the anaerobic digestion (AD) process even because their presence can induce operational problems in the biological reactors (Dolci et al., 2021).

AD of OFMSW has experienced a significant growth in Italy over the last years: about 338 thousand tons of household organics were treated in AD plants in the year 2020 (+35,7% compared to the year 2016) (ISPRA, 2021). OFMSW is characterized by features which can limit successful digestion: high total solids (TS) concentration (15–30%), high carbon to nitrogen ratio (C/N), macro and micro-nutrients' deficiency (nitrogen and trace metals), low buffering capacity, possible presence of toxic compounds (e.g., heavy metals and phthalates) (Maciej Serda et al., 2002; Zhang et al., 2007). Anaerobic co-digestion (AcoD) with various co-substrates is widely used to overcome these issues implying increasing biogas production and more stable process (Iacovidou et al., 2012).

Literature provides few studies centered on the evaluation of the anaerobic degradability of biodegradable plastics. As reported by recent reviews, bioplastics alone are generally not suitable for AD (Abraham et al., 2021; Vardar et al., 2022) although microorganisms are supposed to degrade them. Mater-Bi® (i.e., a biodegradable and compostable bioplastic according to European standard EN 13432) is the material most used in Italy for OFMSW collection bags. What emerges from AD tests is that Mater-Bi® has low biochemical methane potential and is fully recognizable in the digestate at the end of the process (Calabro' et al., 2020; Folino et al., 2020; Vasmara and Marchetti, 2016). This because bioplastic degradation times are often considerably longer than

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conventional hydraulic retention times (HRTs) of AD plants. Better process performances have been achieved either through pre-treatments of bioplastics (Benn and Zitomer, 2018) or AcoD with organic waste (Cucina et al., 2022). To the best of authors' knowledge, most of these studies (of both AD and AcoD of bioplastics) were performed in batch mode with the only aim of assessing bioplastic degradability while there is a lack of investigation on the influence and the fate of bioplastics during semi-continuous AD.

The disposal of wastewater sludge is a problem of growing importance for both the large quantities involved and the increasingly high disposal costs. According to ISPRA (2022), Italian national wastewater sludge production is estimated at around 3 million tons in 2020. As concerned with costs, the disposal of sludge represents up to 50% of the current operating costs of a wastewater treatment plant (WWTP) (Appels et al., 2008). Moreover, due to increasingly strict rules for its final treatment and disposal more sustainable processes have to be found.

In the last years, the need for renewable energy generation and biodegradable waste diversion from landfill have pushed up the role of AD for the treatment of both OFMSW and excess sludge. However, "modern" excess sludge has low methane potential so that making treatment inefficient from a processing and an economic standpoint (Calabrò et al., 2021a; Lim and Fox, 2013). Two main aspects cause low biogas production: Biological Nutrients Removal (BNR) processes that lead to biodegradable matter consumption, and high solids retention times (SRTs) needed for nitrification that induce partial sludge stabilization (Bolzonella et al., 2005). Accordingly, in order to preserve the biodegradable fraction of the influent COD, in "modern" WWTPs the primary settling tank is generally absent. As a result, the specific methane production determined on the secondary sludge (either raw or thickened) is generally in the range 150–300 mL/g_{VS} (Table 1.SI) rather than a typical value of about 400–500 mL/g_{VS} recorded in the past (Metcalf et al., 1991).

In this context, AcoD of excess sludge and OFMSW not only does it allow to turn these waste streams into resources in a perspective of circular economy, but also it solves several technical issues. The Organic Loading Rate (OLR) of many existing AD plants located in WWTPs is often below 1 kg_{VS}/m^3•d because even thickened sludge consists of about 95–98% of water. Conversely, food waste is highly biodegradable and AD plants using it can operate at an OLR of 3 kg_{VS}/m^3•d or even higher (Mata-Alvarez et al., 2014). Hence, the co-digestion can increase the OLR while resulting in a marginal decrease in the HRT. Other important benefits from co-digestion are the C/N balance and the dilution of any inhibitory substances from individual co-substrate (Koch et al., 2016; Mata-Alvarez et al., 2014). Particularly, wastewater sludge with its low C/N can balance the high carbonaceous content of OFMSW so that reaching the ideal range for AD of 15–30 (Del Pilar Anzola-Rojas et al., 2014; Nghiem et al., 2017). AcoD also improves process stability thanks to the high buffering capacity of the sludge that is able to prevent process failure due to rapid acidification typical of food waste. For these reasons, the co-digestion of food waste or OFMSW and wastewater sludge has been extensively applied over the last years resulting in methane yield increase of 20% in comparison with sludge mono-digestion (Borowski et al., 2018).

Taking all these consideration into account, in the present study a simulation at laboratory scale of the semi-continuous AcoD of OFMSW and thickened sludge has been investigated. The novelty of the experiment lies in the presence of Mater-Bi® films representative of bioplastic bags actually adopted for OFMSW collection in a perspective of full-scale implementation. Particularly, the paper aims to: (i) evaluate the influence of Mater-Bi® on methane production; (ii) estimate Mater-Bi® degradation at the end of the process; (iii) assess the phytotoxicity of the final digestate in view of potential agricultural utilization.

Table 1
Characterization of the experimental substrates.

Material	Phase	pH	TS [%]	VS [%TS]	C/N
Digestate	I	7.8	2.5 ± 0.15	67.9 ± 0.77	–
	III	7.5	3.0 ± 0.06	68.5 ± 0.16	–
OFMSW*	I, II, III	6.1	21.7 ± 0.56	96.2 ± 0.13	9.9
Thickened	I	6.2	3.0 ± 0.10	80.6 ± 1.85	6.6
Sludge	I, II	6.5	2.5 ± 0.05	78.2 ± 0.62	
	III	6.2	1.4 ± 0.05	73.4 ± 3.93	
	III	6.4	2.9 ± 0.03	75.2 ± 0.45	
	III	6.5	1.7 ± 0.04	74.8 ± 0.28	
Mater-Bi**	I, II, III	n.a.	97.7 ± 0.35	99.1 ± 0.02	–

*Referred to wet sample before drying, **(Calabro' et al., 2020).

2. Materials and methods

2.1. Inoculum and substrates

The inoculum required to start the AD process was a digestate collected from a full-scale AD plant located in Reggio Calabria province (Calabria Region, Italy) fed with manure and agro-waste. Immediately after collection, it was sieved and pre-incubated at 35 °C before the beginning of the experiment.

Thickened sludge and OFMSW were the main substrates (ingestates) involved in the AD tests. Thickened sludge was collected from a municipal WWTP serving 30,000 people equivalent (P.E.) located in the city of Reggio Calabria (Calabria Region, Italy). The plant operates with a conventional activated sludge system with pre-denitrification and nitrification. The excess sludge is pre-thickened and then subjected to further biological and mechanical treatments. The sludge used in this experiment was collected after the pre-thickening performed by a gravity thickener. Samples of thickened sludge, periodically collected throughout the AD tests, were kept at 4 °C before use.

Different fractions of OFMSW were brought to the laboratory to compose the typical (average) Italian organic domestic waste determined by a recent literature survey (Calabrò et al., 2021b; Calabrò and Pangallo, 2020; Pangallo et al., 2021). Once prepared, the OFMSW was preliminarily dried (at 35 °C for 7 days) and then shredded. Before its use in the AD tests, it was kept at 4 °C. As already mentioned, in Italy, OFMSW is most often collected in Mater-Bi® compostable bags so fragments of these bags were also added to the substrates in order to replicate what occurs during OFMSW treatment at full-scale.

MaterBi® is minimally composed of 60% of starch and starch derivatives and of approximately 40% of synthetic resin that is hydrophilic and biodegradable (Bátori et al., 2018).

Table 1 reports the characterization of the aforementioned inocula and substrates. All the determined parameters (pH, Total Solids (TS), Volatile Solids (VS) and total Volatile Fatty Acids (VFAs)) were measured according to standard methods (APHA et al., 2012; Liebetrau et al., 2016).

2.2. Semi-continuous AD test

The AD tests were performed in semi-continuous mode through a laboratory-scale simulation system (Bioprocess Control Bioreactor, BPC Instruments) involving a total of four glass reactors. Each reactor has a working volume of 1.9 L, it is equipped with an internal stirrer which ensures a complete mixing and it is fully immersed in a thermostatic water bath set at 35 °C. Reactors were fed with input substrates through glass funnels and the discharge of digestate occurred simultaneously. The feeding of the reactors was usually carried out five times per week. The biomethane produced by the AD process was automatically measured by a patented system based on water/gas displacement.

2.3. Experimental design

The experiments have been carried out in three phases: the first was directed at establishing the optimal ratio between thickened sludge and

OFMSW, the second at testing the effect of loading increase and the third for evaluating the eventual effect on methane production and digestate characteristics (i.e. phytotoxicity) specifically induced by Mater-Bi® (Balestri et al., 2019; Menicagli et al., 2019).

In Table 2, quantities of input substrates, involved reactors, and set parameters of the three phases are summarized.

In the first phase, four substrates' ratios were tested (i.e., thickened sludge:OFMSW on VS basis, 100:0, 70:30, 30:70 and 0:100) in four reactors respectively designed as 1, 2, 3, and 4 (Table 2). As already mentioned, along with OFMSW, fragments of Mater-Bi® were also added in a proportion of 2% on VS basis calculated considering the average Italian situation with organic domestic waste. During phase I, the HRT and the OLR were set at 21 days and 1 g_{VS}/L•d, respectively. The duration of the first phase was of 65 days (about 3 HRT).

In the second phase, carried out in continuity to phase I, the feeding conditions of the two best-performing reactors (i.e., reactors 2 and 3) were set equal. Specifically, both reactors were fed with thickened sludge:OFMSW ratio of 50:50 and an OLR of 2 g_{VS}/L•d (HRT was confirmed equal to 21 days, Table 2). The choice of changing substrates' ratios was twofold: on the one hand, maintaining 70:30 (thickened sludge: OFMSW) with a doubled OLR would have implied the feeding of sludge volumes so large that would have been impossible to keep the HRT at 21 days. Moreover, being the purpose of the II phase to evaluate which process better tolerated the OLR increase, it was decided to set equal starting loading conditions. This phase was 42 days long (2 HRT).

Lastly, the third phase of the experiment, aimed at investigating the effects (in terms of methane production and phytotoxicity) of the presence of Mater-Bi® was carried out. This last phase involved two reactors (designed as A and B) which were fed at the same conditions set in the second phase (Table 2). Differently from phases I and II, in this case, fragments of Mater-Bi® were added only in reactor A. The third phase of the experiment lasted 42 days (2 HRT).

2.4. Bioplastic biodegradability assessment during AD and in soil

According to Ruggero et al. (2019) review, in case of Mater-Bi® material, CO_2 measurement, mass loss calculation, spectroscopy, and visual analysis are alternatively or jointly used to assess bioplastic biodegradation under anaerobic conditions. In this study, the biodegradation of Mater-Bi® during the AD processes was evaluated by mass loss calculation and visual inspection. Specifically, the fragments of Mater-Bi® bags added to reactors were progressively numbered according to the day of the feeding. When Mater-Bi® pieces were occasionally collected during digestate discharge or at the end of the AD tests, they were preliminary rinsed with distilled water, dried at 35 °C to constant mass and eventually weighed. Besides, the visual inspection of material erosion (holes, tunnels, etc.) or signs of local disintegration was also carried out.

Furthermore, in order to qualitatively evaluate Mater-Bi® biodegradation in soil, in the case of direct use of digestate as a soil conditioner, at the end of the second phase of AD, tests five pieces of Mater-Bi® per reactor, collected from residual digestates, were covered with commercial gardening soil (Terriccio Universale Compo TerrasanaBio®) and periodically watered under constant conditions of temperature and humidity of about 30 °C and 55%, respectively, for 30 days. In the end, Mater-Bi® pieces were extracted and visually inspected.

2.5. Digestate analyses

pH was directly measured through a digital pH meter (XS instruments) on digestate samples discharged during feeding operations. Furthermore, TS, VS, chemical oxygen demand (COD), ammonium, VFAs, and Volatile Organic Acids/Buffering Capacity ratio (FOS/TAC) were measured on the average weekly samples. Particularly, COD and ammonium were determined by using pre-dosed cuvettes (Merck Millipore COD Cell Test 114,555 and Ammonium Cell Test 114559, respectively) whereas FOS/TAC through a two-point titration (Liebetrau et al., 2016). COD, ammonium, VFAs and FOS/TAC analyses were carried out on the liquid fractions resulting from digestate centrifugation (10,000 rpm for 10 min).

2.6. Phytotoxicity tests

The agricultural quality of anaerobic digestates, in terms of residual phytotoxicity in compliance with the Italian regulation (Decreto Legislativo 29 April 2010, 2010) was assessed by using the comparative germination bioassay of cress seeds (*Lepidium sativum* L.) as proposed by Zucconi et al. (1985) and modified by Di Maria et al. (2014). Briefly, the semi-solid fraction anaerobic digestate obtained by centrifugation was brought to a moisture content equivalent to 85% (wet weight) by adding distilled water and after a contact time of 2 h in the dark at room temperature the suspension was centrifuged (8000 rpm at 4 °C, 20 min) and filtered through a Whatman n 0.42 filter paper. The liquid fraction of the anaerobic digestate (liquor) was directly processed by centrifugation (8000 rpm at 4 °C, 20 min) and filtration (Whatman n. 42 filter paper). Clean water extracts were stored at −20 °C before use. Immediately before the germination assay, clean water extracts were diluted to 30% with distilled water. Seeds of cress were surface-sterilized by soaking in 15% (v/v) NaClO solution for 15 min and then rinsed with distilled water. Ten seeds were evenly distributed into a Petri dish (Ø 9 cm), on a double layer of filter paper previously moistened with 2 mL of each digestate aqueous extract. Distilled water was taken as a control (0%). Petri dishes were then placed in the dark in a growth chamber at 27 ± 1 °C. After 48 h, germinated seeds were counted and their root length was measured by using the ImageJ 1.53e software (National Institutes of Health, USA). The Germination Index [GI (%)] was calculated by multiplying the germination percentage by the root length percentage, divided by 100 as reported by Murillo et al. (1995).

Data from the phytotoxic assay were first tested for deviation from normality (Kolmogorov-Smirnov test) and homogeneity of within-group variances (Levene's test). After running a two-way analysis of variance (ANOVA, reactor mixture × time) to check any significant effect of the substrate, time and their interaction on the variability of the data (the block effect in the experimental design was found to be not significant at $P < 0.05$), multiple pairwise comparison of means was done by Tukey's HSD (honestly significant difference) test at $P < 0.05$ level of

Table 2
Input substrates and set parameters of each phase of the experiment.

Phase	Reactor	Thickened sludge:OFMSW (VS basis)	Thickened sludge [g_{VS}/d]	OFMSW [g_{VS}/d]	Mater-Bi® [g/d]	OLR [g_{VS}/L•d]	C/N	HRT
I	1	100:0	2.0	0.0	0.000	1	9.9	21
	2	70:30	1.4	0.6	0.010	1	7.6	
	3	30:70	0.6	1.4	0.024	1	8.9	
	4	0:100	0.0	2.0	0.035	1	6.6	
II	2	50:50	2.0	2.0	0.035	2	8.3	
	3	50:50	2.0	2.0	0.035	2		
III	A	50:50	2.0	2.0	0.035	2		
	B	50:50	2.0	2.0	0.000	2		

significance. Statistical data processing was done by using SYSTAT 13.0 (Systat Software Inc., Erkrath, D), while all graphs were drawn by using a SigmaPlot v10 software (Systat Software Inc.).

In order to make process flow and sampling more clear, a scheme of the entire experiment is reported (Fig. 1).

3. Results and discussion

3.1. Phase I semi-continuous AD tests

Fig. 2a shows the methane yield during phase I. Two main stages can be identified: a start-up stage lasting about 21 days and, immediately after, the regime stage (duration about 2 HRT). Co-digestion reactors (2 and 3) reported the highest methane production during the entire phase I with two daily peaks: 0.36 NL/g_{VS} (42^{nd} day) and 0.28 NL/g_{VS} (49^{th} day) both recorded in reactor 3 (data not shown). Reactor 1 and reactor 4, fed with thickened sludge and OFMSW only, respectively, presented a different production pattern with a lower average yield. During the regime stage, the average yields recorded were equal to 100 NmL/g_{VS}, 170 NmL/g_{VS}, 190 NmL/g_{VS}, and 92 NmL/g_{VS} for reactors 1, 2, 3, and 4, respectively.

3.2. Phase II semi-continuous AD tests

Phase II involved only reactors 2 and 3 with equal loading conditions. The methane yield, Fig. 2g, during this phase was immediately stable in both reactors despite the increase of organic loading to 2 g_{VS}/L•d with a value of about 0.18 NL/g_{VS}. Thus, no differences between the two processes were recorded during the 2 HRT of total duration of this phase even if the starting conditions (i.e., substrates' ratios of phase I, Table 2) were different.

The increased OLR did not interfere with methane production probably because bacteria were perfectly adapted to loaded substrates.

The present results demonstrate that co-digestion of OFMSW and thickened sludge is a good option for biogas production while using the two substrates alone produces the worst results. It was also shown that the mixture ratios used perform efficiently due to synergetic effects giving improved methane yields compared to the methane potentials of the individual substrates, even doubling the OLR as in phase II experiments. This can be easily seen by comparing measured yields with the weighted average of the yields of sludge and OFMSW alone as shown in Table 3.

In all reactors, during the start-up stage of phase I, pH (Fig. 2b) faced intense variations needing $NaHCO_3$ addition (whenever pH decreased below 6.5, 4 g of $NaHCO_3$ was added to the specific reactor) to increase buffering capacity. Moreover, due to an over-feeding of OFMSW, occurring between the 28^{th} and the 35^{th} day, a reduction is visible in reactors 2, 3, and 4. pH reduction was evident especially in reactors 3 and 4, where the OFMSW percentage was higher while reactor 2 showed minor consequences. Reactor 1 revealed a very stable pH presenting sludge high buffering capacity but also a slow conversion of the substrate. Reactor 4 recorded the lowest pH values and was the reactor needing the most frequent $NaHCO_3$ additions. This behaviour was caused by VFAs accumulation not sufficiently balanced by the alkalinity present. Reactors 2 and 3, after the over-feeding error recovered, and during the regime stage pH was in the optimal range. The pH of both reactors during phase II was stable, except slight variations in the first 7 days (Fig. 2h).

During phase I, TS and VS concentrations (data not displayed) were higher for reactors 1 and 2; reactor 4 had a decreasing trend until week 5 when solid content started to grow reaching a peak during week 7 in correspondence to over-feeding error. In fact, all reactors, except for reactor 1, showed a peak also in the volatile solid content. During phase II, reactors 2 and 3 displayed significant growth in TS and VS concentrations due to the increase of organic loading.

The VFAs concentration and FOS/TAC are two important process

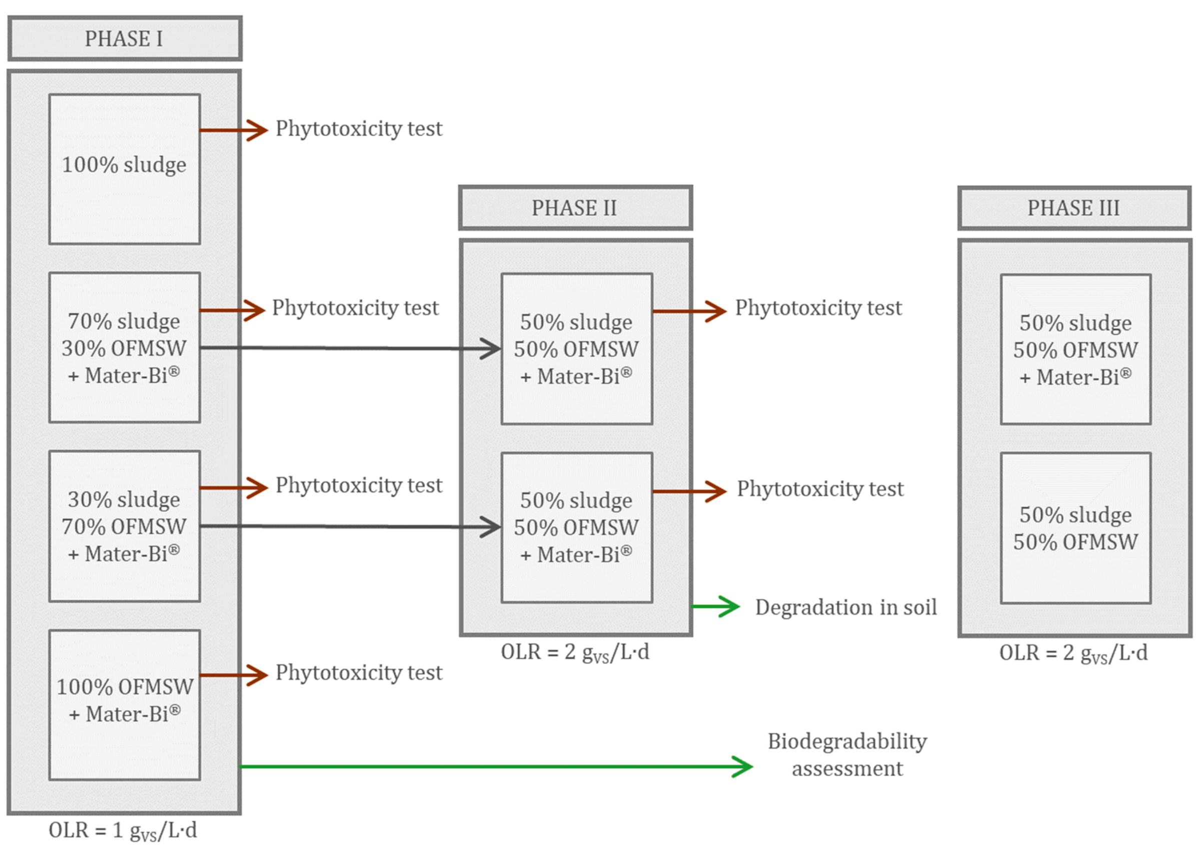

Fig. 1. Scheme of the entire experiment.

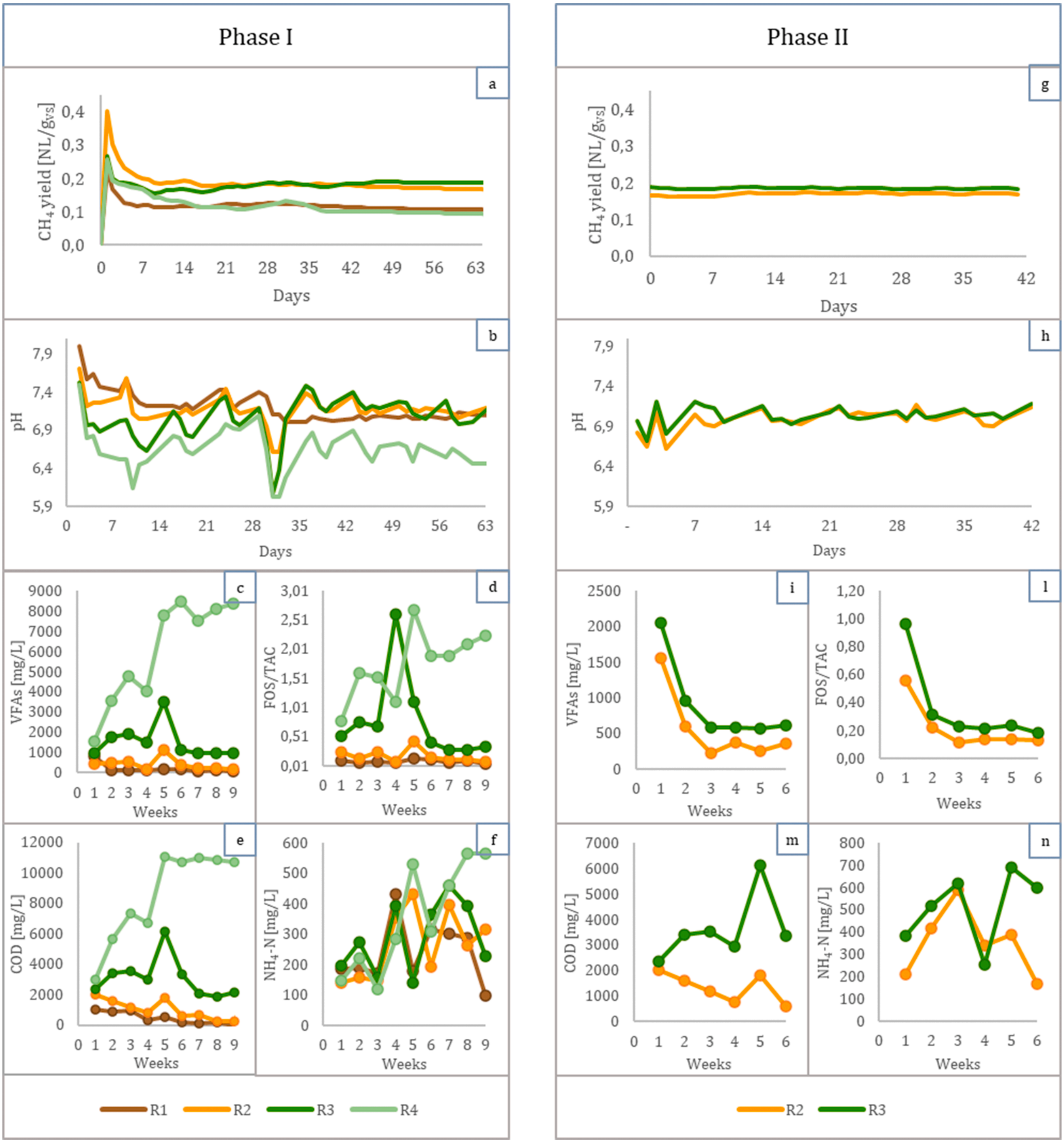

Fig. 2. Semi-continuous results: (a) CH_4 yield, (b) pH, (c) VFAs, (d) FOS/TAC, (e) COD, (f) NH_4-N during phase I; (g) CH_4 yield, (h) pH, (i) VFAs, (l) FOS/TAC, (m) COD, (n) NH_4-N during phase II.

Table 3
Synergic effect of AcoD.

Reactor	Measured Yield [NL/g_{VS}]	% Thickened sludge	% OFMSW	Weighted yield [NL/g_{VS}]
1	0.11	100	0	–
2	0.17	70	30	0.102
3	0.19	30	70	0.097
4	0.09	0	100	–
3–4	0.18	50	50	0.100*

*Calculated by data of phase I.

stability indicators as they indicate the perfect syntrophic interaction among the bacterial strains involved in AD. During phase I, VFAs values (Fig. 2c) seem to be stable until week 5 when, due to the over-feeding error, all reactors showed peaks and then returned to the optimum levels. Observing VFAs concentrations, reactor 4 showed the highest values in the first weeks continuing to have an upward trend due to acids' accumulation. In fact, during the week 5, it increased from 4026.4 mg/L to 7753.4 mg/L. However, the already low methane production had no major modifications. During phase II, reactors 2 and 3 had stable VFAs concentrations (Fig. 2i) after an initial adjustment period.

FOS/TAC values shown in Fig. 2d denote a stable situation for reactors 2 and 3 except for some peaks. Reactor 1 had very low values and this proves again that thickened sludge has low biodegradability. Reactor 4 behaviour demonstrates that the process always worked in an overloaded state. During phase II, the organic loading increase caused a rise in the FOS/TAC values, but reactors 2 and 3 perfectly re-adapted (Fig. 2l). It was noteworthy as both VFAs and FOS/TAC trends were expectedly slightly higher in reactor 3 respect to reactor 2. These behaviours were due to the presence of a larger input quantity in reactor 3 of readily biodegradable sugars (present in the OFMSW) which were progressively converted to acids. In addition, larger volumes of thickened sludge in reactor 2 allowed to buffer acid formation so that keeping FOS/TAC around low levels. Nevertheless, as aforementioned, the response of both reactors (in terms of process stability and methane generation) to the OLR increase of phase II was equal and satisfactory.

The COD concentrations, both during phases I and II, confirmed a progressive stabilization as concerned with reactors 2 and 3 (Fig. 2e, 2 m). Reactor 1 registered the lowest COD values because thickened sludge has a lower organic content. An interesting behaviour emerged from reactor 4 where over-feeding error caused the large rise of the COD concentration as can also be verified by the FOS/TAC ratio.

The ammonium concentration (Fig. 2f, 2n) displays a very irregular trend during phases I and II, but it did not affect FOS/TAC and VFAs values. During phase II, the increase in organic loading caused a growth also of ammonium concentration values, but reactors 2 and 3 showed excellent abilities to adapt. Nevertheless, both in phases I and II, ammonium was always in the range considered stimulating for AD.

Reactors 2 and 3 had a good recovery both after over-feeding error and increased OLR highlighting how the AcoD of OFMSW and thickened sludge performed in a perfect way from a processing standpoint.

3.3. Phase III semi-continuous AD test

The methane yield is reported in Fig. 3a. Reactor A and reactor B needed only a few days to reach stable conditions that were kept ~~during~~ for the whole duration of phase III (2 HRT). They had very similar yields with regime values (about 0.15–0.16 NL/g_{VS}) of about 20% lower than phase II experiments (probably due to the difference in the sludge biodegradability and to the different bacteria adaptation since phase II took place after 105 days in continuity with phase I); it is interesting that methane yield has a slight tendency to rise. From this experiment it becomes clear that the presence of Mater-Bi® fragments has no influence on methane production.

In the first 28 days, pH (Fig. 3b) varied from phase II even if feeding conditions were identical. However, the two reactors behaved similarly and pH tended to increase witnessing a probable progressive adaptation of the microorganisms.

VFAs values (Fig. 3c) were also very similar for the two reactors with a peak around the second week: 1350 mg/L for reactor A and 1081 mg/L for reactor B.

COD concentrations (Fig. 3e) witnessed a progressive stabilization over the course of phase III. Ammonium concentrations (Fig. 3f) were quite irregular for both reactors even if this did not influence the methane production.

3.4. Bioplastic weight loss

The degradation of the Mater-Bi® fragments after AcoD tests (phase I) was studied by weighing pieces before and after the process to record the weight loss according to the procedure reported in Ruggero et al. (2019). Weight loss has been then linked to the permanence of Mater-Bi® fragments in the reactors. Fig. 4 shows no particular trends in all reactors. In most samples weight loss was between 5 and 30% with an average of about 15%.

Bioplastic weight loss was lower than expected especially considering the bioplastic nature. Slightly higher Mater-Bi® mass loss after 28 days and under anaerobic condition was measured by Massardier-Nageotte et al. (2006) (i.e., 45%). In this study, even after a visual

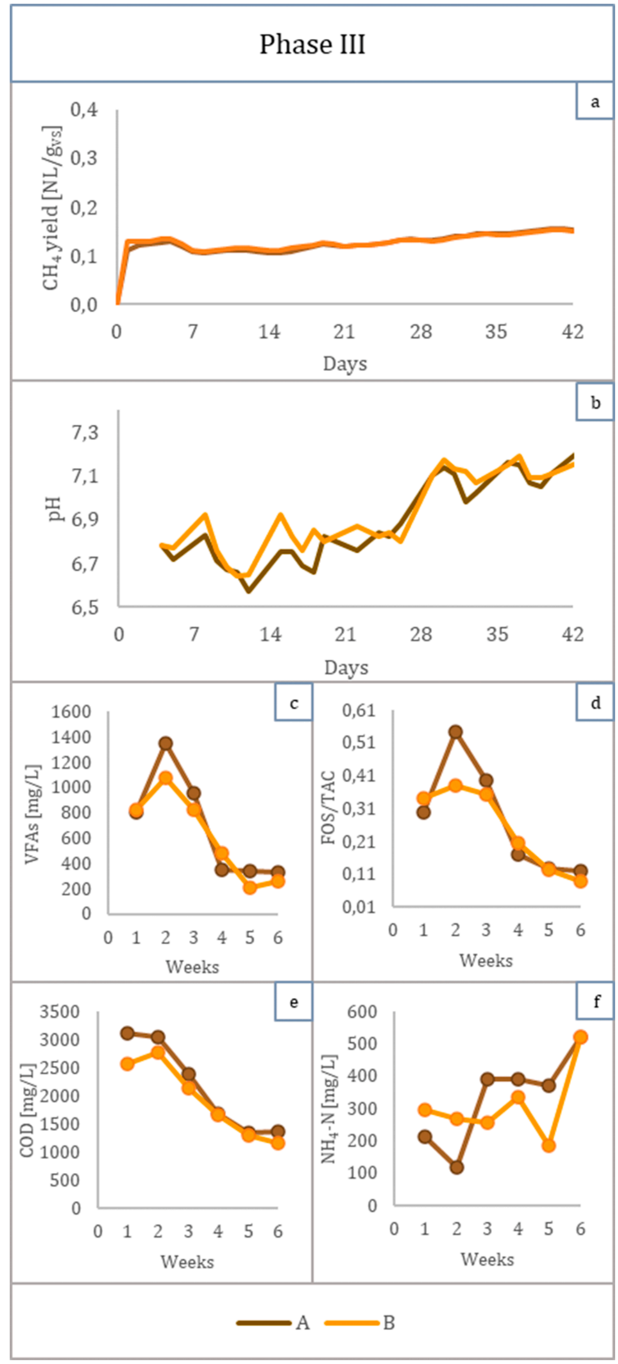

Fig. 3. Semi-continuous results: (a) CH_4 yield, (b) pH, (c) VFAs, (d) FOS/TAC, (e) COD, (f) NH_4-N during phase III.

inspection it is evident that the degradation level was very low. Literature does not report degradation results related to bioplastics during semi-continuous AcoD of OFMSW (containing bioplastics) and thickened sludge, but there are some examples of AD with bioplastics and food waste. Zhang et al. (2018) also stated that the starch-based plastics showed little or no evidence of degradation based on weight loss after AD (Zhang et al., 2019; Zhang et al., 2018).

Degradation in soil was also qualitatively investigated since weight loss was difficult to assess: weight of bioplastics buried in soil for 30 days, rinsed and dried was slightly higher than that recorded before the test. This fact was probably due to an imperfect rinsing and the subsequently incorporation of soil particles. Nevertheless, visually is clear that also they did not degrade significantly since fragmentation did not

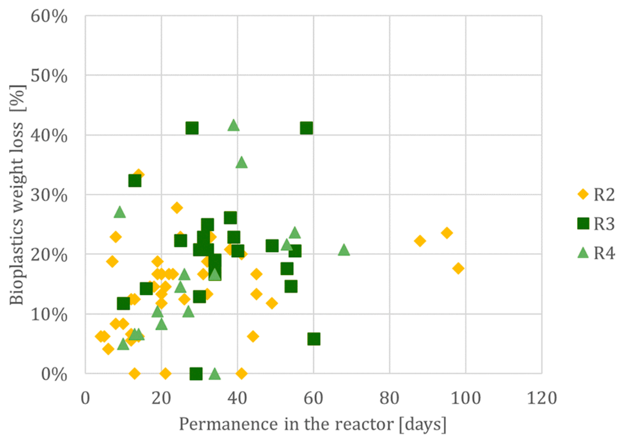

Fig. 4. Bioplastic weight loss after the permanence in the reactors.

occur (Fig. 2.SI).

3.5. Phytotoxicity tests

The *Lepidium sativum* seed bioassay is a widely used, effective and economical procedure to assess the phytotoxic potential of an organic matrix (either composted or not) before its use as a soil conditioner either at field scale or in nursery crop production (Gelsomino et al., 2010). It was found (see Fig. 5) that under all experimental conditions average GI was always lower than the reference limit of 60% according to national regulation (Decreto Legislativo 29 April 2010, 2010), or the minimum acceptable value of 50%, as suggested by Zucconi et al. (1981) and Paredes et al. (2001). This means that as long as digestates show high residual phytotoxicity and immaturity any direct environmental valorization, as for instance, for agricultural application, is severely limited. Needless to say, seed germination and root growth can be both affected by a number of factors, such as the occurrence of heavy metals, ammonia, inorganic salts, low molecular weight organic acids, phenolic compounds, and partially decomposed organic substrates (Bonetta et al., 2014; Said-Pullicino et al., 2007). It is also true that a complete exhaustion of the anaerobic digestion process is rarely achieved (Nkoa, 2014), and post-digestion treatments, including physical, chemical, and biological treatments, are strongly recommended for the production of a safe and renewable fertilizer for agricultural use (Fuchs et al., 2013; Roccotelli et al., 2020).

In this case, the reason for the higher phytotoxicity of the digestate from reactors 3 and especially 4 of phase I can be linked to the presence of ammonium and to the higher VFAs concentration. This fact is corroborated by the statistical analysis that clearly links the higher phytotoxicity to the higher presence of OFMSW or of Mater-Bi® in the substrate. In order to verify this occurrence, phase III experiments have been carried out. It is also worth mentioning that digestate from reactor I fed only with sludge is also phytotoxic but to a lower extent. In this case, phytotoxicity can be attributed both to some component of the digestate and to the occurrence of a high amount of ammonium.

During the experiment of phase III, the phytotoxicity tests were carried out on composite samples of digestate that were collected in weeks 3–4 and 5–6, respectively. Differences of germination index for the tests carried out using the semi-solid sludge were negligible and the index was very low (23% on average for weeks 3–5 and only 0.6% for weeks 5–6). Differences were more noticeable for the liquid fraction. Liquid digestate collected in weeks 3–4 from reactor B (without

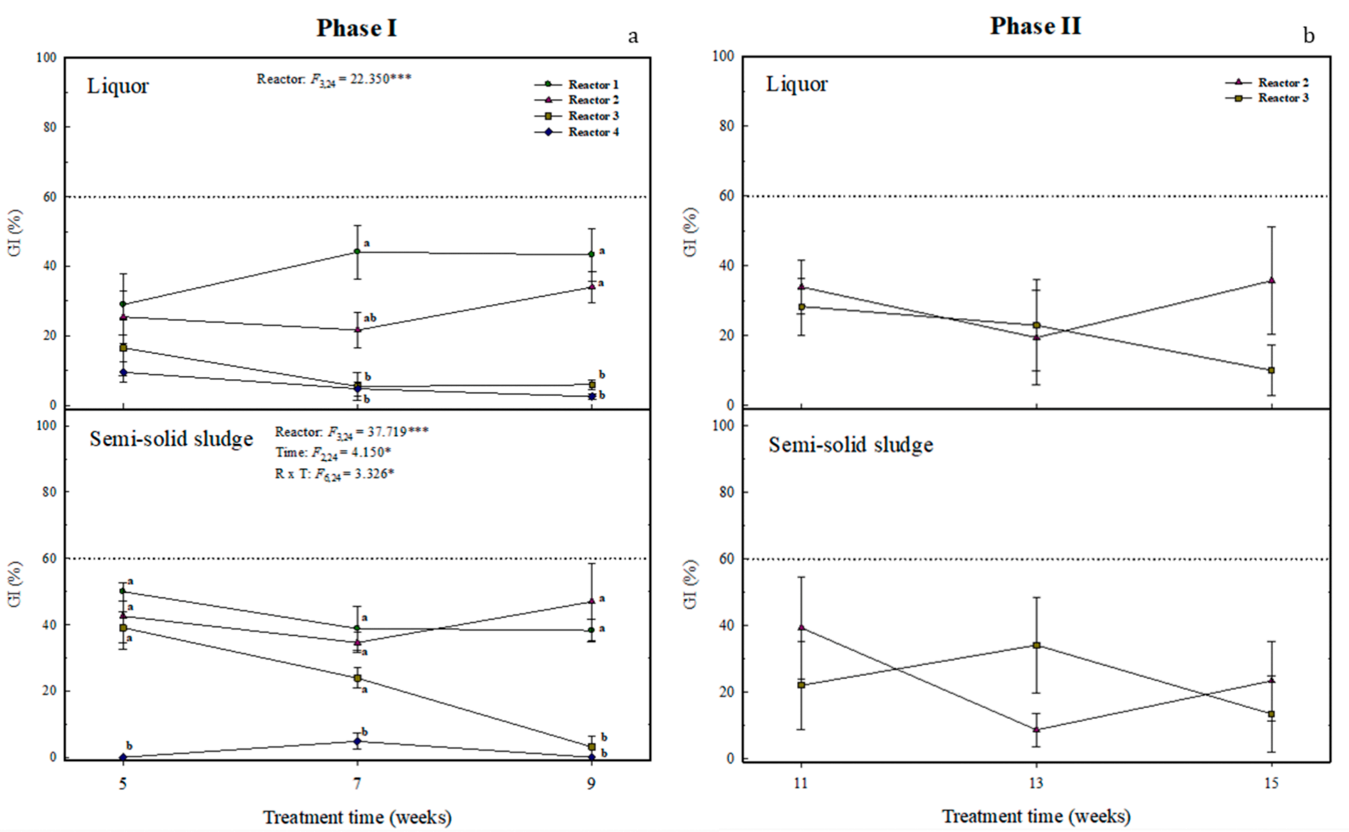

Fig. 5. Variation of the germination index [GI (%)] (mean ± SE, n = 3) of seeds of Lepidium sativum L. during phase I (a) and phase (b). The significant effect due to the reactor (R), time (T) and their interaction (R × T) on the variability of the GI data is shown as F-value and level of significance (* P < 0.05, ** P < 0.01, *** P < 0.001; ns, not significant) estimated by a to two-way analysis of variance (ANOVA, mixture × time). At each sampling time, different letters indicate significant differences among treatments (Tukey's HSD test at P < 0.05).

bioplastics addition) was the only one to be considered non-phytotoxic since the average GI was 67.2% while for weeks 5–6 it decreased to 21.7%. Liquid digestate from reactor A (fed also with Mater-Bi® fragments) was by far more phytotoxic with GI equal to 41.7% and 2.1% for weeks 3–4 and 5–6, respectively. It can be argued that the occurrence of Mater-Bi® induces a higher phytotoxicity in the digestate thus confirming what reported other authors in different conditions (Menicagli et al., 2019). This key point deserves further investigation, possibly using both germination and pot plant assays.

4. Conclusion

The semi-continuous AcoD experiments carried out confirmed that the AD of excess sludge and OFMSW alone is problematic for opposite reasons (yield of about 0.1 NL/g_{VS}); while the co-digestion allowed an almost double methane production (yield of about 0.18–0.19 NL/g_{VS}) and higher process stability. The benefits of AcoD were undoubtedly confirmed even at a doubled loading. From the results of the experiments carried out is certain that Mater-Bi® does not significantly degrade after AD and that does not influence methane production.

However, the feeding of bioplastic bags seems to induce higher phytotoxicity and the presence of undigested fragments could be an issue for further treatment or direct utilization of digestate as soil conditioner. These last issues are worth of further research activity to collect more data on phytotoxicity possibly using different test methods.

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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.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.wasman.2023.05.053.

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