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Comparison of thermophilic anaerobic and aerobic treatment processes for stabilization of green and food wastes and production of soil amendments

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ABSTRACT

The management of organic wastes is an environmental and social priority. Aerobic digestion (AED) or composting and anaerobic digestion (AD) are two organic waste management practices that produce a value-added final product. Few side-by-side comparisons of both technologies and their digestate products have been performed. The objective of this study was to compare the impact of initial feedstock properties (moisture content and/or C/N ratio) on stabilization rate by AED and AD and soil amendment characteristics of the final products. Green and food wastes were considered as they are two of the main contributors to municipal organic waste. Stabilization rate was assessed by measurement of CH_4 and CO_2 evolution for AD and AED, respectively. For AD, CH_4 yield showed a second-order relationship with the C/N content ($P < 0.05$); the optimal C/N ratio indicated by the relationship was 25.5. For AED, cumulative CO_2 evolution values were significantly affected by the C/N ratio and moisture content of the initial feedstock ($P < 0.05$). A response surface model showed optimal AED stabilization for a C/N of 25.6 and moisture of 64.9% (wet basis). AD final products presented lower soluble chemical oxygen demand (COD) but lower humification degree and aromaticity than the products from AED. This lower stability may lead to further degradation when amended to soil. The results suggest that composting feedstocks with higher C/N produces an end-product with higher suitability for soil amendment. The instability of end products from AD could be leveraged in pest control techniques that rely on organic matter degradation to produce compounds with pesticidal properties.

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1. Introduction

Annual worldwide municipal solid waste (MSW) generation has been estimated to be 1.7–1.9 billion metric tons, with organic material being the most abundant type of waste (e.g. food waste, wood, and yard trimmings) (Chen et al., 2016). The main methods used for MSW management are landfilling, incineration, composting, and anaerobic digestion (AD) (Chen et al., 2016; Patil et al., 2016). Composting is the most popular technique to recycle organic wastes (EPA, 2013). During composting, a consortium of microbes degrades organic matter (OM) under aerobic conditions in both thermophilic and mesophilic environments with the thermophilic stage of the process being the most active (Marshall et al., 2004; Sharma et al., 1997). Compost is valued for its pathogen-free OM content and its capacity to enhance chemical, physical and biological properties of soils (Gajalakshmi and Abbasi, 2008). Many biotic and abiotic factors control the performance of composting processes including aeration, moisture content, and C/N ratio (Chang and Hsu, 2008; Golueke, 1972; Guo et al., 2012; Kumar et al., 2010; Torres-Climent et al., 2015; Zhu, 2007). Carbon serves primarily as an energy source for the microorganisms, while a small fraction of the carbon is incorporated into microbial cells. Nitrogen is essential for microbial growth, as it is a constituent of protein that forms over 50% of

Abbreviations: AD, anaerobic digestion; AED, aerobic digestion; BMP, biomethane production; CER, CO_2 evolution rate; cCER, cumulative CO_2 evolution; COD, chemical oxygen demand; CP-MAS NMR, cross-polarization magic angle spinning nuclear magnetic resonance; DS, digestate sludge; FSP, fiber saturation point; FW, food waste; GW, green waste; MSW, municipal solid wastes; OM, organic matter; VFA, volatile fatty acids; VS, volatile solid.

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dry bacterial cell mass and is a critical element in enzymes involved in waste hydrolysis (Yu et al., 2017). A C/N ratio between 25 and 35 is the optimal range for composting feedstock (Golueke, 1992; Guo et al., 2012). Moisture content is also a key factor during composting as it affects microbial activity and mass transfer within the biomass (Gajalakshmi and Abbasi, 2008; Guo et al., 2012; Torres-Climent et al., 2015). Water solubilizes the nutrients required by microorganisms and most of the decomposition occurs in the thin liquid film on the surface of solid organic particles (Tseng et al., 1995). Although optimal moisture content depends on the stage of the composting process and the feedstock, an initial moisture content between 50–70% is generally considered ideal for composting (Guo et al., 2012; Haug, 1993).

Biogas production through AD of organic wastes is a market that is expanding significantly (Pazera et al., 2015). AD is a technology where a consortia of microorganisms produces biogas through decomposition of organic matter in the absence of oxygen (Khalid et al., 2011). The advantages of using AD are that it recovers some of the energy inherent in the original material and offers the possibility to recycle nutrients (Nielfa et al., 2015). AD can be performed at low solids content (Total Solids, TS < 15%) or high solids (TS > 15%) and under thermophilic (55 °C) or mesophilic (37 °C) conditions (Khalid et al., 2011). Thermophilic AD has been reported to be more efficient in decomposing organic wastes and destroying pathogens than mesophilic AD (Shi et al., 2013). Most studies recommend an operating C/N ratio range of 20 to 30 with an optimal ratio of 25 for anaerobic bacterial growth in an AD system (Yan et al., 2015). However, the optimal C/N ratio varies with the type of feedstock to be digested (Khalid et al., 2011; Shi et al., 2013).

The different microbial communities involved in composting and AD will have different impacts on the final products. Composting typically has greater microbial activities and higher decomposition rates of cellulose and hemicellulose and higher cell protein production compared to AD (Lin et al., 2014). In contrast, microbial communities active in AD typically have greater conversion of crude protein to ammonia compared to composting (Lin et al., 2014). Furthermore, compared to mesophilic digestion, thermophilic digestion has significantly higher holocellulose degradation due to more favorable conditions for cellulolytic and xylanolytic microorganisms (Lin et al., 2014; Shi et al., 2013).

One of the main added-value final products from composting (compost) or AD (anaerobic digestate) is soil amendment. The impact of compost and digestate amendment on soils has been extensively studied and reviewed (Gajalakshmi and Abbasi, 2008; Nkoa, 2014; Tambone et al., 2013; Tambone et al., 2015). Compost is used more than anaerobic digestate for soil amendment because of the low phytonutrient content and high organic matter quality of compost (Tambone et al., 2007). Digestates have been suggested to have better potential for use as fertilizers compared to compost because AD may promote the preservation and concentration of inorganic nutrients such as P, K and N (Lin et al., 2014; Nkoa, 2014). However, anaerobic digestates may pose an environmental risk associated with potential NH_3 emission and high Mn, Cu and Zn concentrations that can induce toxicity in agricultural soils following repeated applications (Nkoa, 2014).

When using the same feedstock, AD and AED may yield products with differing stability due to microbiota and bioconversion pathways that are unique to each approach. As more municipalities collect and treat food and green waste and combine them in composting and AD processes, it is important to understand the outcome of both processes on product quality. Information about the quality of the stabilized product will inform management strategies for organic wastes. Highly stable products are normally recommended for soil amendment. On the other hand, lower stability products might be leveraged in integrated pest management strategies. For instance, soil biosolarization and anaerobic soil disinfestation are techniques that rely on decomposition of amended organic matter to generate heat and/or biopesticidal fermentation products to inactivate soil borne pathogens (Achmon et al., 2016; Hewavitharana and Mazzola, 2016; Simmons et al., 2013). To our knowledge, no side-by-side studies of both processes using the same initial feedstock have compared their performance and assessed the final product properties for suitability of use as soil amendment. This study aimed to assess the impact of the initial feedstock properties (moisture content and C/N ratio) on stabilization rate and characteristics associated with final products. Studies were completed in laboratory bioreactors to measure stabilization under low solids anaerobic digestion or under high solids aerobic conditions (composting) at different initial moisture contents. Maturity and stability of the final products were measured to assess their potential use as soil amendments.

2. Material and methods

2.1. Initial feedstocks

The feedstocks were two model green (GW) and food wastes (FW, Table 1). The material composition of the GW was based on the California 2008 Statewide Waste Characterization Study (Yu et al., 2017). The GW components and mass fractions included leaves (41.0% dry basis), grass (11.4% dry basis), prunings and trimmings (40.4% dry basis), and branches and stumps (7.2% dry basis) (Yu et al., 2017). Dog food was selected as a model FW to avoid the confounding effects of heterogeneous composition and particle size that are often encountered in municipal food waste (Baker et al., 1999). The particular dog food used was selected based on its compositional similarity to food wastes measured in a municipal waste management facility in Dubai (data not shown). The main composition of the FW was: 22% Protein, 14% Fat, 50% Carbohydrate and 13% Crude Fiber (Oral Care Adult Dog Food, Hill's® Science Diet®, USA). The inoculum used in the AED and AD processes was a thermophilic liquid digestate from an anaerobic digester located on the University of California, Davis campus (UC Davis). The digester processes mixed organic waste (food, agriculture, and green wastes) at thermophilic conditions (55 °C), and at low solids loading rate (5–10% of total solids).

The feedstock and inoculum were the same for both types of digestion although the inoculation level and water content varied between the AD and AED processes. The feedstocks were mixed at different levels to obtain different initial C/N ratios. The experimental C/N ratios were estimated from the measured C/N of the GW and FW. C/N values of 17, 20, 23, 27 and 34 were achieved by preparing GW/FW ratios (g/g dry basis) of 21/79, 35/65, 50/50, 67/23 and 100/0, respectively.

2.2. Experimental set-up for AD experiments

Batch anaerobic digesters shown elsewhere (Achmon et al., 2016) consisted of 250-mL glass media bottles fitted with modified caps connected to an in-line check valve (catalog #80103, Qosina, Edgewood, NY). These digesters permitted headspace gas to leave the digester without risk of oxygen contamination from retrograde airflow (Achmon et al., 2016). Digesters were loaded with 7.5 g (dry weight) of GW/FW mix and 92.5 g of digestate sludge as inoculum (DS, wet basis, Table 1). Anaerobic digestion experiments were completed at moisture contents greater than composting experiments because digestion was not successful at low moisture contents. Three replicates per feedstock treatment were used. Following preparation, digesters were incubated at 55 °C until methane production ceased (14–15 days). Methane and carbon

Table 1
Properties of the model food and green wastes.

	Dry matter %	C total %	N total %	C/N	Ash %	VS %	Fiber Saturation Point (FSP)[a] %
FW	91.5	45.5	3.7	12.4	5.3	94.7	410
GW	92.1	48.8	1.4	34.1	4.7	95.3	472
DS	3.5	1.2	0.34	3.6	–	–	–

[a] 100 * g water/g dry weight.

dioxide content in the biogas produced from each digester was measured with a MicroOxymax respirometry system (Columbus Instruments, Columbus, OH) according to the manufacturer's instructions for anaerobic operation. Total biogas production for each digester was estimated by summing the measured volumes of evolved carbon dioxide and methane, the primary constituents of biogas. Methane quality was approximated as the ratio of the volumetric fraction of CH_4 and CO_2 in the biogas.

The methane evolution data from AD were using to predict the biomethane potential (BMP) using a Modified Gompertz model (Nielfa et al., 2015)

$$BMP = BMP_{max} \times \exp\left(-\exp\left(\frac{k(\lambda - t)e}{BMP_{max}} + 1\right)\right) \quad (1)$$

where γ (ml CH_4/g VS) is the maximum volume accumulated at an infinite digestion time (t), k is the specific rate constant (ml CH_4/g VS/d) and λ is the lag phase time constant (days). This equation is a modification of the Gompertz model and assumes biogas production is proportional to microbial activity. Parameters were estimated by fitting cumulative methane production as a function of time to Eq. (1).

2.3. Experimental set-up for composting experiments

The composting experiments were completed to examine both the influence of C/N and moisture content on stabilization rate. The C/N ratios used were the same as previously described. The experimental water content target was approximately 60% (low, L), 73% (medium, M) and 85% (high, H) of the fiber saturation point (FSP), which was equivalent to 62%, 69% and 74% moisture content (wet basis). To estimate the FSP, feedstocks were soaked in water for an hour and then the excess water was left for drainage for another hour. The moisture content of the saturated feedstock was then measured by weighing the wet samples before and after drying at 105 °C for 24 h. The FSP was expressed as the percentage of the mass of water held by a given mass of feedstock (dry weight).

As with the AD system, the inoculum for composting experiments was DS. This was done to ensure the initial microbial community was similar for both experiments. To confirm that DS was stable, aerobic bioreactors loaded with only DS were run in parallel with the samples and the CER was negligible (data not shown). The inoculum levels for the low, medium and high moisture content studies were 5%, 8% and 10% of the initial feedstock (dry mass equivalent), respectively. These differences were attributed to the high moisture content of the DS (3.5% total solids), so inoculum amount was adapted to the target moisture content. To maintain the initial moisture content during the composting experiments, distilled water was added to the reactors every 3–4 days.

A detailed description of the aerobic system can be found elsewhere (Reddy et al., 2013). Prior to loading the bioreactors, the feedstock, inoculum and distilled water were thoroughly mixed to reach the target moisture content. The 250-mL bioreactors (four replicates) were loaded with 6 g (dry weight) of each mixture. To maintain aerobic conditions, reactors were supplied with air at a rate of 20 mL/min and were incubated at 55 °C in a temperature-controlled incubator. As for the AD experiments, incubation was stopped after the maximum respiration rate was achieved and the cumulative respiration approached steady-state (approx. 14–15 days). In the composting system, the respiration rate in terms of CO_2 evolution rate (CER) and cumulative respiration (cCER, mg CO_2/g dw) were monitored as stabilization indicators. From these values, the maximum cumulative respiration ($cCER_{max}$, mg CO_2/g dw) was estimated by fitting cumulative respiration as a function of time to an exponential model as expressed in Eq. (2).

$$cCER = cCER_{max} \times (1 - e^{-kt}) \quad (2)$$

where k is a rate constant (d^{-1}).

2.4. Analysis of the final products from anaerobic and aerobic digestion

For analyses of the final products, the C/N of 17 was excluded as the composting process failed and respiration stopped within a few hours of initiating incubation. Therefore, the final material could not be considered as representative of composted material. For the same reason, in the AED samples, only the samples from the 60% FSP treatments were considered. As reference of the initial state of the feedstock, the non-digested inoculated feedstocks prepared for the composting experiments were used (initial). All samples were air-dried prior to analysis.

2.4.1. Chemical characteristics of the water extracts from the final products

The electrical conductivity (EC), pH, chemical oxygen demand (COD) and Volatile Fatty Acids (VFAs) of the samples before and after incubations were determined in water extracts prepared from 1:20 (mass of sample dry weight/mass of water) mixtures of solid samples and distilled water that were equilibrated for one hour at room temperature. The pH and EC were measured using a pH meter (Mettler Toledo, Columbus, OH, USA) and a conductivity meter (Mettler Toledo, Columbus, OH, USA) according to the manufacturer's guidelines. For COD and VFA analyses, extracts were centrifuged for 10 min at 10,000 g. The COD was determined using 0.5 or 0.1 ml of the supernatant and the reactor digestion method kit (COD TNTplus Vial Test, HR, 20–1500 mg/L, Hach Company, Loveland, CO).

For VFA analysis, an aliquot of the supernatant was filtered through a 0.2 μm filter (Titan-3, 17 mm filter blue 0.2 μm PTFE membrane, Thermo Fisher Scientific Inc. San Diego, CA, USA) into an HPLC vial. Acetic, propionic, formic, valeric, isovaleric, butyric and isobutyric acids were measured using an HPLC-UFLC-10Ai (Shimadzu, Columbia, Maryland USA) equipped with an Aminex® HPX-87H (300 × 7.8 mm) column (Life Science Research, Education, Process Separations, Food Science, Hercules, California USA) and a SPD-M20A diode array detector set at 210 nm. The HPLC conditions are described elsewhere (Simmons et al., 2016).

In the AD experiment the COD and the total N content were monitored in the liquid sludge at the end of the incubation. In this case, 0.02 and 0.005 mL of the sludge were used for COD and total N analyses, respectively. The total N was measured using the TNT Persulfate Digestion Method kit provided by Hach (Hach Company, Loveland, CO).

2.4.2. UV spectroscopy of compost samples

The UV spectroscopy of final product extracts was used to assess the degree of maturity and stability following an adapted method (Sellami et al., 2008). A mixture of 0.1 g of air-dried sample and 50 mL of 0.5 M NaOH was shaken for 2 h and then centrifuged at 1238 g. An aliquot of the extract was diluted 1:1 with distilled water for analysis. The absorbance spectrum between 200 and 830 nm was recorded on a Eppendorf BioSpectrometer (Eppendorf, NY, USA). The absorbance (E) of the solution at the wavelengths of 664 nm (E_6), 472 nm (E_4) and 280 nm (E_2) was measured. The absorbance at 280 nm corresponds to non-decomposed lignin, aliphatic and quinone moieties; absorbance at 472 nm is associated with OM in the initial actively decomposing stage, and 664 nm is associated with organic material with a high content of aromatic and condensed functional groups, denoting stable humified material (Sellami et al., 2008; Zbytniewski and Buszewski, 2005).

The ratios E_2/E_4, E_2/E_6 and E_4/E_6 were calculated to describe various humification phenomena. The ratio E_2/E_4 was used as an indicator of the relative amounts of lignin at the beginning of humification. The ratio E_2/E_6 was employed to relate non-humified and highly humified material (Fuentes et al., 2006; Sellami et al., 2008). Finally, the E_4/E_6 ratio was used to assess humification degree.

2.4.3. Organic matter characterization with Nuclear Magnetic Resonance

^{13}C-Solid State Cross Polarization Magic Angle Spinning Nuclear Magnetic Resonance (^{13}C-CPMAS-NMR) was used to characterize the main functional group of the organic C in the samples. Data were collected using a Bruker AVANCE500 NMR spectrometer equipped by a widebore 11.74 Tesla magnet. The Larmor frequency for ^{13}C resonance is 125.76 MHz. The samples were loaded into 4 mm zirconia rotors and spun at 15 kHz. A cross-polarization pulse sequence with variable amplitude contact power and two-pulse-phase-modulation decoupling was used for data acquisition. The contact time was 2 ms, and the recycle delay time was 2 s. A total of 2048 transits were used for signal averaging. The functional group classification of the organic C was as follows: (i) aliphatic-C bonded to other aliphatic chain-Short Chain (0–27 ppm); (ii) aliphatic-C bonded to other aliphatic chain-Long Chain (27–47 ppm); (iii) O and N-alkyl C including methoxyl carbon and N-substituted alkyl carbon in protein (47–113 ppm); (iv) aromatic-C phenol or phenyl ether-C (113–160 ppm), and (v) Carboxyl-C keto-C (160–210) (Piterina et al., 2009; Tambone et al., 2015). To estimate the stability of the substrates, the aromaticity index was calculated based on the ratio of the integrated areas of C types as in Eq. (3) (Piterina et al., 2009):

$$\%\ \text{Aromaticity} = \frac{\%\ \text{Aromatic}(113-160\ \text{ppm})}{(\%\ \text{Aliphatic}(0-47\ \text{ppm}) + \%\ \text{Alkyl}(47-113\ \text{ppm}) + \%\ \text{Aromatic}(113-160\ \text{ppm}))} \times 100 \quad (3)$$

2.5. Statistical analysis

Factorial and response-surface analyses, regression, one-way ANOVA and Tukey's Honest Significant Difference (HSD) post hoc tests were performed using JMP-IN software (version Pro 12, SAS, Cary, NC). The significance level was set at 0.05. The models were optimized using the minimum least square method considering the leverage effect. Second order and interaction effects were included in the analyses.

3. Results and discussion

3.1. Effect of C/N ratio of the feedstock on the anaerobic digestion rate

The efficacy of the anaerobic digestion process was assessed by monitoring CH_4 and CO_2 evolution and estimating the BMP. After 14d of incubation, the peak of methane production had been achieved and the rate of methane production began to decline for all samples (Fig. 1A). At this point, the amount of CH_4 recovered corresponded to more than 85% of CH_4 estimated as BMP for each C/N, meaning that at this point most of the potential CH_4 was already produced. Treatments with a C/N of 34 and C/N of 17 yielded the lowest BMP values (67.46 and 50.45 mL CH_4/g VS, respectively, Fig. 1B, Table S1). Mixtures with C/N ratios of 20 and 27 exhibited an average BMP of 113.01 and 118.51 mL CH_4/g VS, respectively. The maximum BMP (185.19 mL CH_4/g VS) was observed at a C/N ratio of 23. The one-way ANOVA analysis showed significant differences ($P < 0.05$) between some of the treatments. Specifically, the HSD-Tukey analysis showed that methane production at C/N ratio of 23 was significantly higher than at C/N ratio of 34 ($P < 0.05$) and C/N 17 ($P < 0.05$). Regarding the quality of the biogas produced (Fig. 1B, Table S1), the best quality, assessed by the ratio CH_4/CO_2, was observed for the C/N ratio of 23 (1.96, that is, 66% of the biogas was CH_4) and the lowest quality was observed for the C/N ratio of 34 (1.39, where only 59% of the biogas was CH_4). Differences in the biogas quality between the different C/N ratios were not significant.

The estimated BMP_{max} showed a significant second order polynomial relationship with the C/N ratio ($P < 0.05$). The fitted model using least minimum squares ($R^2 = 0.73$, Table S3) was:

$$BMP_{max} = -710.9 + 67.9(C/N) - 1.33(C/N)^2 \quad (4)$$

Based on this model the optimal C/N ratio for these feedstocks would be 25.5 producing up to 156 mL CH_4/g VS. This estimated C/N ratio would correspond to a green waste/food waste ratio of 61/39 (g dry weight/g dry weight).

The final COD and total soluble N values for the liquid digestate provided information on the stability of the anaerobic digestion process. Both parameters showed a significant linear negative correlation with the C/N ratio (Fig. 2, $P < 0.05$ and $P < 0.05$ for N and COD, respectively). The sample with the C/N ratio of 17 showed the lowest degradation rate and the highest COD and N for all treatments. Although high N concentration may be a result of higher hydrolysis efficiency during anaerobic digestion, it has also been observed that unbalanced C/N ratios can result in elevated total ammonia nitrogen release and/or elevated volatile fatty acids accumulation that are intermediates but also potential inhibitors of the AD process (Yan et al., 2015). AD inhibitors may have slowed the digestion rate or inactivated microorganisms central to the digestion process, which could explain the higher COD at the C/N of 17.

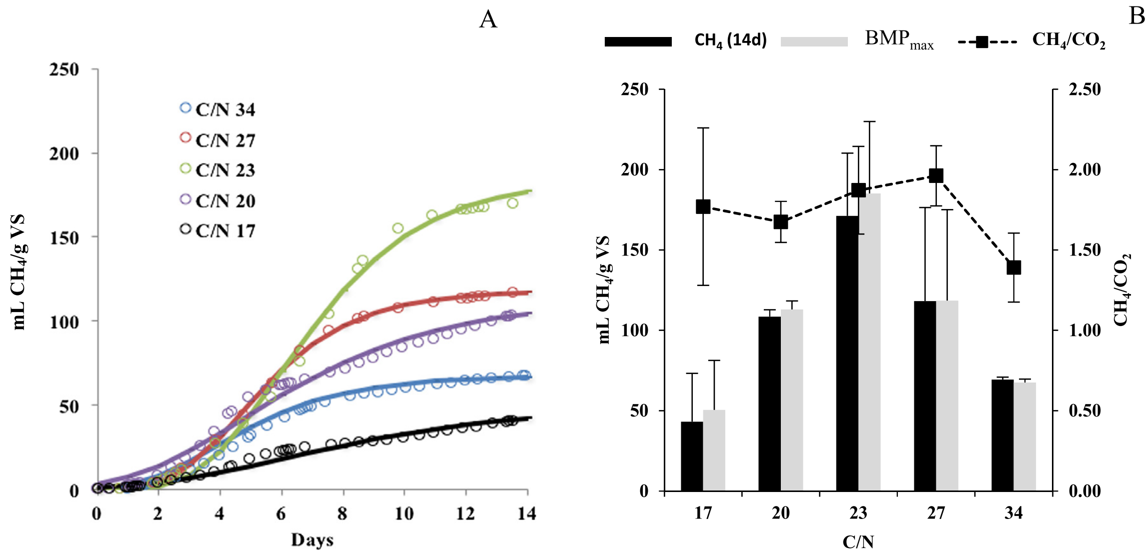

Fig. 1. (A) Mean (n = 3, points) cumulative and predicted (full line) CH_4 evolution (mL CH_4/ g Volatile Solid) at different C/N ratios. (B) Cumulative CH_4 emission after 14 days, estimated maximum biomethane potential (BMP_{max}), and biogas quality assessed by the CH_4/CO_2 ratio (right axis).

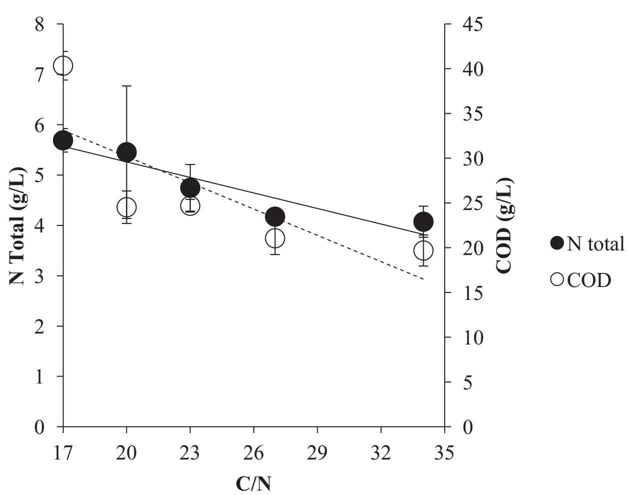

Fig. 2. Total N (g/L, left axis) and COD (g/L, right axis) of the liquid digestates after anaerobic digestion as a function of initial C/N ratio. Bars represent the standard deviation (n = 3) and lines represent the linear relationship between dependent variables and initial C/N ratio.

3.2. Effect of C/N ratio and moisture content on the aerobic digestion process

Treatments experienced variable cumulative respiration values depending on the C/N ratio and moisture content (Fig. 3A). For the same moisture content (60% of the FSP), the lowest cCER was for the treatments with C/N ratios of 17 and 34, whereas the highest cumulative respiration was recorded at the C/N of 20. In general, performance of treatments with lower C/N ratios was affected by excess water; microbial activity ceased after 24–48 h of incubation for mixtures with C/N ratios of 17, 20 and 23 at the highest moisture content tested (Fig. 3A). Treatments with a C/N ratio of 17 had no detectable activity at any moisture content while treatments with a C/N ratio of 20 failed at 70% of the FSP. Conversely, the same mixture at 60% of the FSP, presented the highest cCER for all samples tested (1231.79 mg CO_2/g dry weight). Treatments with GW only (C/N = 34) had a cCER of 489.74 and 226.64 mg CO_2/g dry weight at 80% and 60% of the FSP, respectively (Table S2).

Guo et al. (2012) also observed that a high moisture content and a low C/N ratio restricted organic degradation (Guo et al., 2012). The initial moisture content in composting is important as it provides mobility to microorganisms and helps with nitrogen mineralization and polysaccharide hydrolysis. Too high moisture content can fill the small pores leading to limited oxygen transport and generation of anaerobic conditions (Hubbe et al., 2010). Moreover, for food waste (low C/N treatments) the hydrolysis of substrates can result in the accumulation of liquid further decreasing air-filled porosity. The lower respiration rate for the GW at all tested moisture contents could be attributed to both limited available N and/or recalcitrant lignocellulosic material in the woody fraction of GW (Zhang et al., 2016). Additionally, the cellulosic material from the GW acts as bulking material and provides a more porous structure for gas diffusion that can prevent N loss (Bernal et al., 2009). At the C/N ratio of 17, there was likely less porosity in the biomass due to a lack of GW bulking agent. This may have prevented oxygen from permeating the biomass, leading to anaerobic conditions that inhibited the composting process (Gajalakshmi and Abbasi, 2008).

A response-surface analysis of the $cCER_{max}$ as a function of the moisture content (expressed as moisture content, MC, %wet basis) and the C/N ratio showed a significant relationship ($P < 0.05$ and a $R^2 = 0.59$, Eq. (5), Table S3).

$$cCER_{max} = -13765 + 140(C/N) + 398(MC) + 4.92(C/N)(MC) - 8.98(C/N)^2 - 4.02(MC)^2 \quad (5)$$

The $cCER_{max}$ for AED had significant first-order and second-order relationships with C/N ratio ($P < 0.05$ for both), a linear relationship with moisture content ($P < 0.05$), and an interaction between C/N and moisture content ($P < 0.05$). According to this model, optimal composting conditions would be achieved at C/N of 25.6 and a moisture content of 65% to produce a $cCER_{max}$ of 947 mg CO_2/g dry weight.

3.3. Effect of the conversion process on the characteristics of the final products

3.3.1. pH, EC, VFAs and COD levels of the final products

The C/N ratio of the initial non-digested substrates was positively correlated with the pH ($P < 0.05$) and negatively correlated

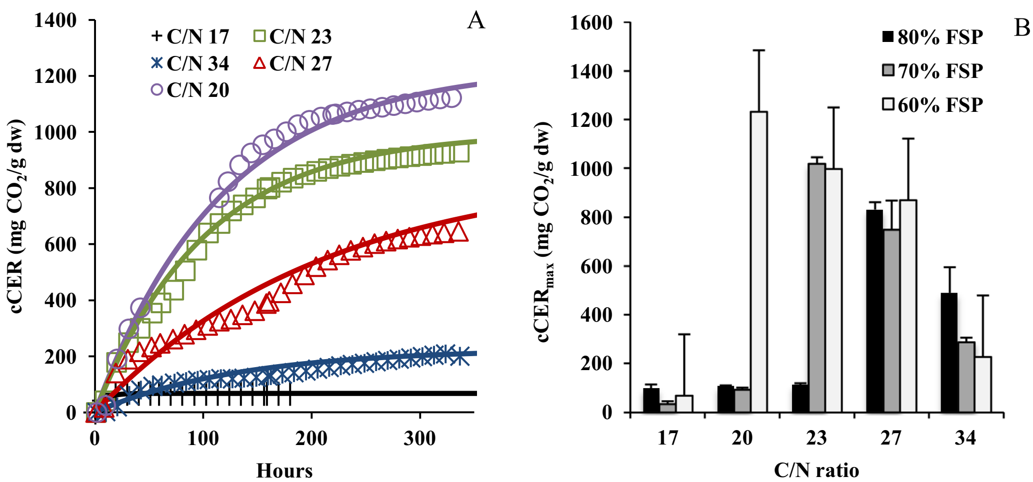

Fig. 3. (A) Cumulative CO_2 evolution (cCER, mg CO_2/g dry weight) of feedstocks incubated at 60% of the fiber saturation point (FSP) at different C/N ratios. (B) Estimated maximum cCER as a function of C/N ratio.

Table 2
Mean pH, electrical conductivity (EC) and chemical oxygen demand (COD) of the initial feedstocks and final solid products from anaerobic digestion (AD) and composting at different C/N ratios.

Treatment	C/N	pH*	EC* mS/cm	COD* mg/g
Initial	20	5.87 ± 0.10a	2.96 ± 0.36b	211.60 ± 22.00b
	23	6.15 ± 0.26ab	2.69 ± 0.12ab	201.80 ± 19.52 ab
	27	6.10 ± 0.12bc	2.87 ± 0.43ab	195.80 ± 21.21ab
	34	6.44 ± 0.06c	2.15 ± 0.18a	142.00 ± 4.28a
AD	20	7.81 ± 0.25a	3.62 ± 0.16a	66.69 ± 8.25a
	23	7.95 ± 0.03a	2.97 ± 0.12b	45.07 ± 1.53b
	27	8.13 ± 0.02a	2.94 ± 0.10b	43.01 ± 6.00b
	34	8.08 ± 0.03a	2.29 ± 0.07c	37.81 ± 3.33b
Compost (60%FSP)	20	6.96 ± 0.10a	3.79 ± 0.30a	108.18 ± 5.79a
	23	7.10 ± 0.16a	3.21 ± 0.24b	83.16 ± 7.51b
	27	7.17 ± 0.07a	2.50 ± 0.05c	62.68 ± 5.11c
	34	6.59 ± 0.27b	1.52 ± 0.05d	64.18 ± 1.93c

* Different letters indicate significant differences (P < 0.05) within the same treatment.

with the EC and COD (P < 0.05, Table 2). The samples from solid anaerobic digestates had a pH ranging from 7.12 at C/N of 17 to 8.08 at C/N of 34 (Table 2). A slight but significant linear correlation was found between the C/N ratio and the pH (P < 0.05). In contrast, the EC showed a negative linear correlation with the C/N (P < 0.05) decreasing from 3.90 mS/cm at the C/N ratio of 17 to 2.29 mS/cm at the C/N of 34 (Table 2). The pH of the composted samples ranged from 6.59 to 7.17 (Table 2). The treatments with a C/N ratio of 34 showed a significantly lower pH compared to the other treatments (P < 0.05). As for the anaerobic digestates, a strong negative correlation was observed between the EC and the C/N ratio (P < 0.05); the EC decreased from 3.79 mS/cm at a C/N ratio of 20 to 1.52 mS/cm at a C/N of 34.

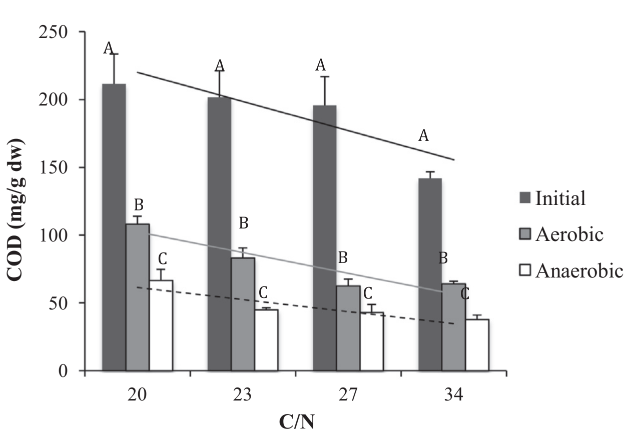

Fig. 4. Mean chemical oxygen demand (COD) before digestion (initial), and after 14 days of anaerobic and aerobic digestion. Lines represent significant linear relationship between the COD and the C/N ratio (P < 0.05) for the initial (black), aerobic (grey) and anaerobic (dotted) samples. Different letters indicate significant differences among the samples within the same C/N (P < 0.05).

The soluble COD can provide insight into the stability of the biomass and be used as an indicator of the initial available carbon in the solid products (Thomas et al., 1996). The non-digested samples had a significant negative correlation with C/N ratio (P < 0.05) ranging from 142 mg/g for samples with a C/N ratio of 34 to 211 mg/g for samples at a C/N of 20 (Fig. 4). Anaerobic digestion and composting significantly reduced the COD of the samples at every C/N ratio. After anaerobic digestion, the COD of solids also correlated negatively with the C/N (P < 0.05), decreasing from 67 at a C/N of 20 to 38 at a C/N of 34. A similar significant trend was observed for the composted material (P < 0.05), decreasing from 108 (mg/g) to 64 (mg/g) at C/N ratios of 20 and 34, respectively. Finally, when comparing AD and composting, the composted substrates always showed significantly higher COD values (P < 0.05) than the solids from AD at each C/N ratio.

Table 3
Mean volatile fatty acid content in the initial feedstocks and solid final products from anaerobic digestion (AD) and composting at different C/N ratios.

Treatment	C/N	Formic mg/g	Acetic mg/g	Propionic mg/g	Butyric mg/g	Valeric mg/g
Initial	20	3.49 ± 0.96b	–	5.52 ± 0.37	–	–
	23	5.42 ± 5.30b	1.77 ± 3.07	5.71 ± 2.45	–	–
	27	20.56 ± 8.04a	–	12.13 ± 7.18	–	–
	34	31.45 ± 4.51a	–	4.73 ± 3.08	0.21 ± 0.37	–
AD	20	0.56 ± 0.46b	0.81 ± 0.23	1.18 ± 1.00	0.39 ± 0.22	3.51 ± 2.65
	23	1.14 ± 0.17b	0.84 ± 0.06	2.17 ± 1.81	0.84 ± 0.31	0.72 ± 1.25
	27	2.46 ± 1.63ab	0.34 ± 0.59	0.01 ± 0.02	0.34 ± 0.42	1.73 ± 1.03
	34	4.78 ± 1.07a	0.40 ± 0.69	–	0.65 ± 0.32	0.64 ± 0.34
Compost (60%FSP)	20	–	–	7.53 ± 0.95	–	–
	23	–	–	7.84 ± 1.72	–	–
	27	6.17 ± 11.96	–	6.28 ± 4.19	–	–
	34	1.17 ± 0.61	–	5.15 ± 9.37	0.57 ± 0.87	–

*Different letters indicate significant differences ($P < 0.05$) within the same treatment. No letter indicates no significant difference.

The initial substrates contained formic and propionic acids. Substrates with C/N ratios of 27 and 34 showed significantly higher concentrations of formic acid compared to other substrates. The high initial concentration of formic acid in the GW feedstock (C/N of 34) and the linear correlation with the C/N ratio ($P < 0.05$), indicate that GW already has these acids naturally. After AD, the VFAs detected in the solid anaerobic digestates were formic, acetic, propionic, butyric and valeric acid. Formic acid was highest at the C/N ratio of 34 (4.78 mg/g), and showed a significant negative linear correlation ($P < 0.05$) when the C/N ratio decreased (Table 3). On the other hand, acetic acid showed a significant negative correlation when the C/N ratio increased ($P < 0.05$). The variety of VFAs observed in the composted samples was lower compared to AD samples. Only propionic, formic and traces of butyric acids were detected. Propionic acid was found at levels between 5–10 mg/g for every treatment. The statistical analysis did not show any significant trends or differences between samples at different C/N ratios.

3.3.2. UV spectroscopy of the final products

UV absorbance was used to determine the degree of humification in the final product extracts. In general, when comparing the E_4/E_6 ratio of the initial samples (Fig. 5), the solids remaining from samples incubated in anaerobic conditions showed higher values than the initial samples and the samples incubated in aerobic conditions showed lower values than the initial samples. Particularly, the E_4/E_6 value of the aerobically digested samples at the C/N ratio of 27 was significantly lower than the solids from samples incubated under anaerobic conditions. The E_2/E_6 ratio showed similar trends as the E_4/E_6 ratio. No significant differences were observed in the E_2/E_4 ratio between the different C/N ratios. The E_2/E_4 ratio reflects the proportion between the lignins and other materials at the beginning of humification, and the content of feedstock at the beginning of transformation. The generally higher E_2/E_6 and E_4/E_6 values observed for the AD final products compared to AED products indicates lower stability in the products from AD. In addition, the E_2/E_6 and E_4/E_6 ratios showed a linear positive correlation with the C/N ratio ($P < 0.05$). This also suggests that the degree of stability increases with increasing C/N ratio. Finally, for soil humified material, the typical E_4/E_6 ratio is less than 5 (Chen et al., 1977). Other compost studies have found values around 8 or higher (Zbytniewski and Buszewski, 2005). The larger E_4/E_6 values for this study indicate that stabilization was incomplete. Managing the stability of soil amendments is very important for agricultural practices as there may be some risks associated with application of unstable amendments. For instance, it has been observed that application of unstable organic matter can increase soil organic matter loss (Teutscherova et al., 2017) and enhance nitrogen loss (Moral et al., 2009). For this reason, based on the results from this study, products from feedstocks with higher C/N, which would require shorter treatment times, would be recommended for cases in which highly stable materials are needed for amendment to soil.

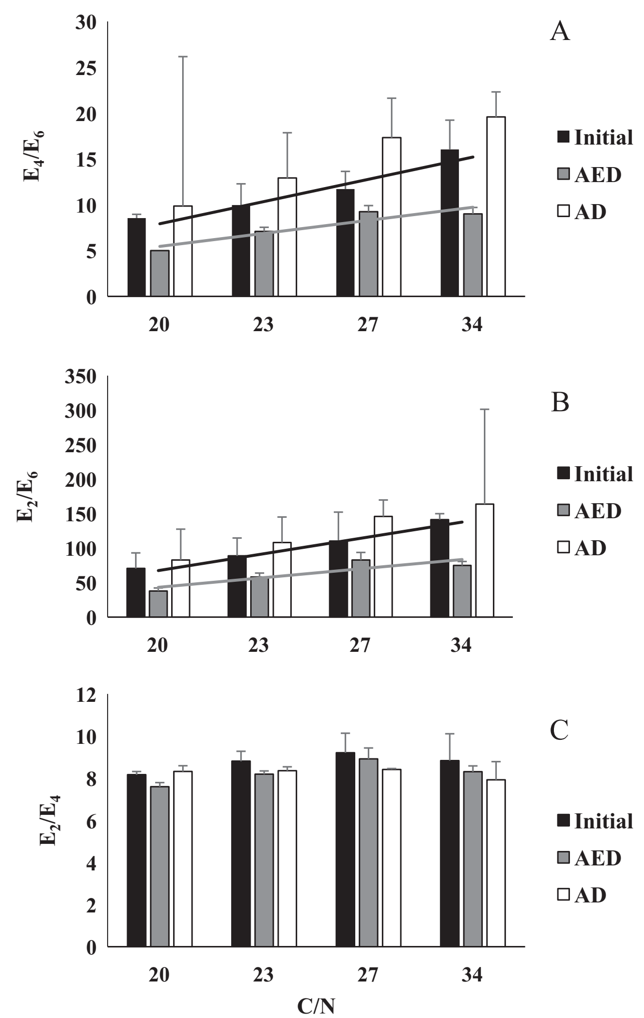

Fig. 5. UV absorption E_2/E_4, E_2/E_6, E_2/E_4 ratios before digestion (initial), and after 14 days of anaerobic (AD) and aerobic digestion (AED). Lines represent significant linear relationship between the absorption ratio and the C/N ratio ($P < 0.05$) for the initial samples and final products.

3.3.2.1. ^{13}C-CP-MAS NMR spectroscopy analysis of the final products. The solid products from AD and AED at C/N ratios of 34, 27 and 20 were analyzed in solid state ^{13}C-CP-MAS NMR to obtain qualitative and semi-quantitative information on the molecular features of the solid state of the OM.

The Aliphatic C region (0–45 ppm) of the samples represented between 10–21% of the organic carbon (Table S4, Fig. 1SI). This region is associated with proteins, lipids, and aliphatic branched and short-chain molecules (Piterina et al., 2009; Tambone et al., 2015). The spectra show two large peaks in this region at 24 ppm (short chain branched aliphatic C) and at 30–33 ppm (the methylenic C in the long chains of aliphatic compounds, such as suberin, cutin, waxes and fatty acids) (Piterina et al., 2009). The O and N-alkyl C (45–110 ppm) region is primarily associated with O-substituted alkyl carbon in carbohydrates (Piterina et al., 2009), while the peak at 174 ppm (160–200 ppm) includes carboxylic acids primarily associated with organic acids that are free of esters or amides (Piterina et al., 2009). The predominant peak was found at 72–74 and 82 ppm, which is associated with O-alkyl-C of atoms C-2, C-3 and C-5 in polysaccharides (cellulose and hemicellulose) (Piterina et al., 2009). This peak, along with the peak at 105 ppm that represents the atoms of the anomeric carbon (C-1) of cellulose (Kogel-Knabner, 2002), indicates that hemicellulose and cellulose are the dominant material at any C/N ratio, before and after digestion. The signals observed at 56 ppm (lignin methoxyl-C) and the region between 130–153 ppm (aromatic C ring) are related to aromatic C from lignins and lignin-derived molecules (Tambone et al., 2015). These signals increased after digestion indicating an accumulation of these recalcitrant compounds. In agreement with UV-spectroscopy results, composted material and samples with elevated C/N ratios presented higher accumulation of lignins. Lignins have been shown to be correlated with the cation exchange capacity (CEC) of the organic matter (Bernal et al., 1998). The CEC is an important soil property as it improves the capacity of the soil to store plant nutrients and the buffering capacity of soil pH (Peverill et al., 1999). For this reason, products originating from feedstocks with elevated C/N and products from composting would be recommended for agricultural practices that require pH buffering and plant nutrient storage.

Fig. 6 presents a comparison of the percentages of the different organic C groups after anaerobic or aerobic digestion relative to the initial feedstock. Total aliphatic chains showed a general decrease after digestion. The opposite trend was observed for aromatic C, which increased for all the digested samples. This increase was 90% and 81% for the aerobic and anaerobic digested samples, respectively, at C/N of 20. For the same C/N ratio, the composted samples always showed a greater change than samples produced under anaerobic digestion. On the other hand, the carboxyl groups were reduced in all the samples except for the samples at C/N ratio of 34 from aerobic digestion. In this case, carboxyl groups were more reduced after anaerobic digestion compared to aerobic digestion for the same C/N ratio, being 60% lower for the C/N of 20 after anaerobic digestion. The increase in aromatic C and phenols might be related to the degradation of non-aromatic cell wall compounds, which leads to a relative enrichment in aromatics (Tambone et al., 2013). This is due to the preference of microorganisms to decompose easily degradable C compounds resulting in accumulation of recalcitrant molecules. The dominance of more recalcitrant compounds in the digested samples is confirmed by the increase in aromatic carbon at 56 ppm that corresponds to lignin methoxyl-C (Dignac et al., 2000).

The aromaticity values are also known to provide an indication of the evolution of humification during composting (Albrecht et al., 2008). The aromaticity increased for every digested sample. For a specific initial C/N ratio, the aromaticity was higher for the composted material than for the products from AD. Although UV-spectroscopy results suggested that the stability of the material was not complete, both UV-spectroscopy and ^{13}C-CP-MAS NMR results suggest that for the same initial feedstock, aerobic digestion yielded more stable and humified products than AD. The critical influence of humic substances on the physical, chemical, and biological properties of soil has been extensively demonstrated (Canellas and Olivares, 2014; Diacono and Montemurro, 2010). This indirect effect on soil properties along with the direct specific structural and physiological responses of plants to humic material have been shown to promote plant growth (Canellas and Olivares, 2014; Diacono and Montemurro, 2010).

In summary, the results from this study suggest that if the intended goal of the treatment process is a product with relatively higher humic content and stability, that waste management specialists would manage feedstocks to obtain a C/N between 27–34 and process said feedstocks under aerobic conditions. Such processing would avoid amendment of unstable organic matter to soil and prevent the accumulation of VFAs that may negatively impact soil health (Poggi-Varaldo et al., 1999; Tunes et al., 2013). However, certain agronomic soil disinfestation practices, like soil biosolarization and anaerobic soil disinfestation, can leverage instability of organic matter. The efficacy of these practices is based in part upon the microbial activity associated with the decomposition of unstable organic matter amendments which produce VFAs that can control pests (Fernández-Bayo et al., 2017b; Hewavitharana and Mazzola, 2016; Momma, 2015; Simmons et al., 2013). These practices may also serve as a post-treatment step for unstable amendments to improve soil quality. For instance, previous studies

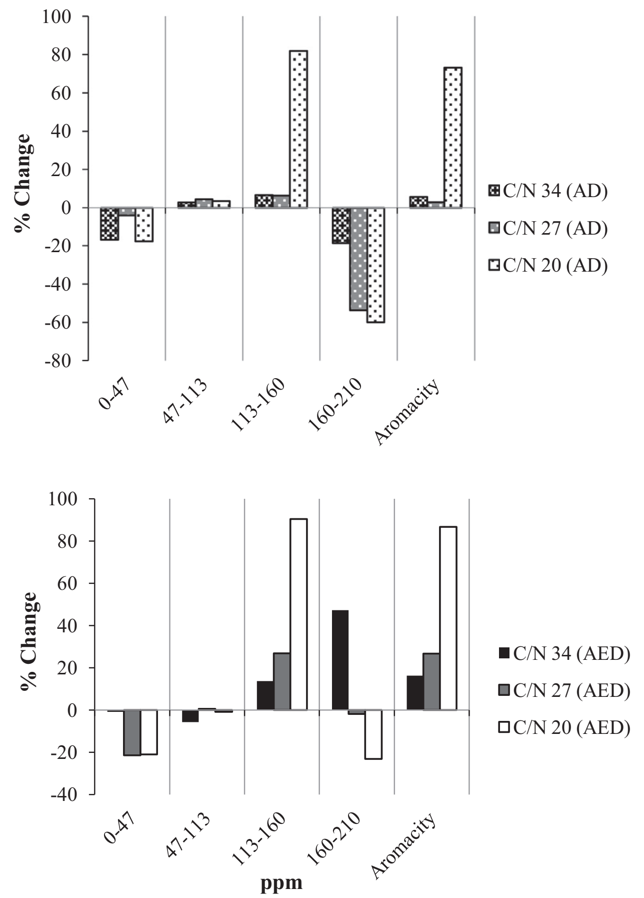

Fig. 6. Percent change in total aliphatic-C (0–47 ppm), O-alkyl carbon (47–113 ppm); aromatic carbon (113–160 ppm) and carboxyl groups (160–210 ppm) for the solid anaerobic and aerobic final products compared to the initial feedstock at the C/N ratios of 34, 27 and 20.

have shown a positive impact on plant-available water, total C, extractable P and K and microbial biomass in digestate-amended soils that were treated by soil biosolarization (Fernández-Bayo et al., 2017a; Fernández-Bayo et al., 2017b).

4. Conclusions

Despite being different biological processes, similar optimal C/N ratios (25.6) were found for aerobic and anaerobic digestion. Initial moisture content played a more significant role for composting of substrates at C/N of 17 compared to a C/N of 34. This is of importance for organic waste management decision makers in regions where water is a scarce resource. The composted material showed more bioavailable organic material compared to material that was anaerobically digested based on COD. The properties of the OM of the final products indicated a consistently lower stability for solids from AD and, within the same digestion process, at lower C/N ratios. The most stable and humified OM was observed for samples with C/N ratio between 27–34 and treated under aerobic conditions. This stable OM would be suitable for application as soil amendment to improve soil quality. The lower stability and/or higher COD in products could be leveraged in soil-borne pest control techniques that require unstable substrates to promote accumulation of biopesticidal VFAs. Further investigation is needed on whether higher COD or lower stability degree are more efficient to control pests.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.wasman.2018.05.006.

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