This is a pre-print of an article published in Waste and biomass valorization (Ed. Springer). The final authenticated version is available online at: https://doi.org/10.1007/s12649-016-9743-6

The effect of a short term aerobic pretreatment step on the anaerobic co-digestion of the organic fraction of municipal solid wastes: Liquid extract addition versus solid phase addition

Francisco Martínez-Valdez [1,3], Dimitrios Komilis[2], Gerardo Saucedo-Castañeda[1], Raquel Barrena*[2], Antoni Sanchez[2]

[1]Biotechnology Department, Metropolitan Autonomous University Campus Iztapalapa (UAM-I), México D.F. Iztapalapa, 09340.

[2]Composting Research Group (GICOM), Department of Chemical, Biological and Environmental Engineering, Escola d'Enginyeria, Universitat Autònoma de Barcelona (UAB), Cerdanyola del Vallès, 08193 Barcelona, Spain.

[3]Chemical Engineering Division National Mexico Technology Campus Chimalhuacán México, Estado de México, 56330

* Corresponding author. Tel: +34 93 581 10 18, Fax: +34 93 581 20 13, E-mail: raquel.barrena@uab.cat

Keywords: Anaerobic digestion; OFMSW; co-digestion; enzymes; aerobic pretreatment

Abstract

Goal of the work was to study the effect of mixing aerobically pretreated organic municipal solid wastes (OFMSW) with raw OFMSW in an anaerobic digestion process. The optimum time of aerobic pretreatment was found to be five days, as this was indicated via biological activity measurements (oxygen uptake rate, enzymatic activities, temperature). The aerobically pretreated wastes or a liquid extract from those pretreated wastes were, each separately, mixed with simulated OFMSW in various experiments. The mixtures were anaerobically digested for 28 days and 5 different treatments were performed including the blanks. The methane generation results were fitted to a typical anaerobic model to calculate theoretical maximum methane potential, maximum methane generation rate and theoretical lag time. Results indicated that the addition of 5-day aerobically pretreated OFMSW in solid form to raw OFMSW resulted in a 18% net increase of the methane production. The R_{max} was also increased by 39% while no significant differences in the lag time of the methanogenic phase were observed. The addition of the liquid extract that was obtained from the 5-day pretreated OFMSW did not result in a statistically significant increase of the net methane production of the raw OFMSW.

1. Introduction

Anaerobic digestion (AD) of organic materials is a treatment technique that aims to valorize wastes via the generation and exploitation of biogas. In recent years, much effort has been made in the implementation of anaerobic digestion to treat the Organic Fraction of Municipal Solid Waste (OFMSW). Therefore, the principal obstacle to the wider spread of anaerobic digestion technology in solid wastes (as opposed to liquid wastes) is the relatively low rate of biodegradation, due to this limiting solids (mainly composed of lignocelluloses) hydrolysis step (Mata-Alvarez et al., 2000).

Biological pretreatment includes both anaerobic and aerobic methods, as well as the addition of specific enzymes, such as peptidase, carbohydrase and lipase, to the anaerobic digestion process. Aerobic pretreatment, such as composting, can be an effective method to obtain a higher hydrolysis of complex substrates due to the higher production of hydrolytic enzymes, which is induced by the increased specific microbial growth (Ariunbaatar et al., 2014, Güelfo et al., 2011). Furthermore, hydrolysis step can also be improved through the increase in the microbial activity per unit of surface area. This effect can be achieved not only by substrate inoculation, but also by the use of enzymes directly. Therefore, biological pretreatments include both the use of microorganisms with high ability in degrading a substrate and the addition of enzymes that support biological reactions within anaerobic digesters (Cesaro & Belgiorno, 2014). However, an extensive aerobic biodegradation step can oxidize most of the biodegradable carbon under aerobic conditions, rendering the remaining organic material a substrate with a relatively low biogas yield, despite the faster establishment of methanogenesis (Gerassimidou and Komilis, 2013). According to the results obtained by Brummeler and Koster (1990), a composting pretreatment of OFMSW

resulted in a 19.5% volatile solids (VS) loss. Actually it is not clear in the literature what is the distinction between the short and long periods of pretreatment that can distinguish between these two different effects of aerobic pretreatment on the pretreated material (i.e. a) material with higher biogas yields and a faster establishment of methanogenesis versus b) semi-stabilized material with a significantly lower biogas yield). An effort to clarify that distinction had been recently investigated by Gerassimidou and Komilis (2013).

In addition to the above, there is a lack of information on whether an extract obtained from the aerobically pretreated OFMSW, which is expected to be rich in hydrolytic enzymes, can also affect biogas production when added to a solid substrate. Both the mixing of different solid waste substrates and the addition of liquid extracts to raw OFMSW can be considered co-digestion processes. The co-digestion of OFMSW with other co-substrates such as vegetable oils, manure or straw for instance has been demonstrated to significantly enhance biogas production (Ponsá et al., 2011; Abudi et al., 2016; Tian et al., 2015, Yong et al., 2015). The benefits of the co-digestion process are: dilution of potential toxic compounds eventually present in any co-substrates involved; adjustment of moisture content and pH; increased content of biodegradable material; expanding the range of bacterial strains involved in the process (Mata-Alvarez et al., 2014; Álvarez et al., 2010). However, the obvious benefits of adding liquid extract, rich in enzymes, to raw MSW is that smaller anaerobic digesters can be built compared to when directly adding a solid aerobically pretreated co-substrate.

Based on the above, the main goal of the experimental work was to investigate the effect of introducing: i) OFMSW which was aerobically pretreated over a short period, and

ii) enzymatic extract obtained from the same aerobically pretreated OFMSW, in the anaerobic digestion (AD) process of raw OFMSW.

For this aim, several lab-scale anaerobic digestion experiments were performed to quantify biogas and methane yields, as explained in section 2. The degradation process of an 11-day aerobic pretreatment step was followed by measuring the oxygen uptake rate (OUR) and the enzymatic activities during the process. This was done to quantify the extent of aerobic degradation as well as the time of the peak biological activity. This aided in establishing the optimum time of pretreatment. The pretreated material removed at that optimum time was used as a co-substrate during the anaerobic experiments that followed. The co-substrates were either the same pretreated material, in its solid form, or a liquid extract obtained from that aerobically pretreated solid waste.

2. Materials and Methods

2.1. Substrates

Source selected OFMSW was obtained from an industrial composting plant near Barcelona, Spain. OFMSW was collected already mixed with pruning waste (a bulking material), in a volumetric ratio 1:1. Simulated OFMSW was prepared by following a recipe that took into account a typical composition of raw OFMSW as suggested by the Agència de Residus de Catalunya (2006). This composition (on a wet weight basis) was: 17% cooked pasta, 7% bread, 15% salad components, 17% tomatoes, 17% apples, 17% oranges, 7% cooked meat and 1% napkins.

The inoculum used in the anaerobic digestion experiments was digested OFMSW in the form of slurry obtained from a full-scale OFMSW anaerobic digester (Barcelona, Spain). The inoculum had a moisture content of 88.61% ± 0.53%, (wb), a volatile solids (VS) content 58.64% ± 0.37% (db) and a pH of 8.1.

Aerobically pre-treated OFMSW was obtained from 10 L reactors on the 5th day of the process performed in the laboratory (with simulated OFMSW). This day was selected since the material was observed to have the maximum biological and enzymatic activity at that time (as was observed after having performed 11 day aerobic experiments in the same reactors). In addition to the solid material, liquid extracts were obtained from the aerobically pretreated OFMSW to use as a co-substrate, instead of pretreated solid waste. The extracts refer to the soluble part that was extracted from the corresponding pre-treated OFMSW samples by orbital shaker at 200 rpm for 20 min. using a ratio of 1 g sample per 5 mL of water. The initial characterization of the materials is shown in Table 1.

2.2. Experimental procedure

At a first place, the fresh OFMSW obtained from a municipal composting plant was aerobically degraded in lab scale experiments to determine the time that the peak biological activity (based on the Oxygen Uptake rate, OUR) is achieved. The pretreated OFMSW, directly in the form of solid, or the liquid extract obtained from that solid, were mixed with simulated raw OFMSW (S-OFMSW) in the subsequent anaerobic digestion (AD) experiments. The reason that simulated OFMSW were used is that they can provide a rather consistent and reproducible material that can overcome the inherent heterogeneity of the raw OFMSW that commonly leads to a large variance among replicate runs.

The experimental design was developed according to Table 2. As shown in Table 2, the aerobically pretreated OFMSW and the corresponding pretreated extract were used as co-substrates with simulated raw OFMSW. Control runs with artificial OFMSW, solid pretreated OFMSW and the extract from pretreated OFMSW were performed to allow the comparison between the Biochemical Methane Potential (BMP) of the OFMSW with and without the use of the selected co-substrates. BMP from raw OFMSW from composting plant was also determined with the aim to quantify the loss of biogas potential during aerobic pretreatment.

2.2.1. Aerobic biodegradation experiments

The aerobic degradation experiments were undertaken during 11 days. Measurements of oxygen consumption, enzymatic activity, fatty acid concentration, protein concentration and reducing sugars concentration were performed. The aim was to obtain a complete profile of the aerobic degradation process in order to determine when the maximum degradation activity would be produced. This period of maximum activity was selected as the aerobic pretreatment time prior to the anaerobic digestion (AD) experiments.

The aerobic degradation experiments of the OFMSW were performed in 10 L custom made sealed stainless steel reactors (20 cm diameter, 36 cm height). These were filled up with 6 kg of the material and performed in triplicates. The reactors were equipped with temperature, airflow and oxygen monitoring and online calculation of the specific oxygen uptake rate (sOUR). This value was calculated as the difference in oxygen content of input and output airflow per amount of dry matter present in the reactor, following Equation 1:

$$sOUR = F\ (0.209 - yO_2)\ \frac{P \times 60 \times 30}{R \times T \times DM} \qquad \text{(Eq. 1)}$$

where sOUR is the specific oxygen uptake rate (g O_2 kg^{-1} DM h^{-1}); F, the airflow in the reactor (L min^{-1}); yO_2 , is the oxygen molar fraction in the exhaust gases (mol O_2 mol^{-1}); P, the pressure of the system that was assumed constant at 101,325 Pa; 32 is the oxygen molecular weight; 60 is the conversion factor from minute to hour; R, the ideal gas constant (8310 Pa L K^{-1} mol^{-1}); T, the temperature at which F is measured (K) and DM, the dry matter of material placed in the reactor (kg). Total cumulative consumption (ATu) was determined through the continuous OUR data obtained during the experiments.

The experiments were performed under near-adiabatic conditions with continuous aeration at a minimum rate of 0.1 L/min. The reactors included a data acquisition system with a PLC (programmable logic controllers), which allowed data reading every minute. Particularly, PLC system read the values of oxygen, airflow and temperature, which are connected to a personal computer, and it enables on-line complete monitoring. The oxygen was regulated by means of airflow manipulation in the exhaust gas to maintain the system in favourable aerobic conditions (oxygen content above 12%), as previously described (Puyuelo et al., 2010).

2.2.2. Anaerobic biodegradation test

Anaerobic batch tests were developed following the procedure described by Ponsá et al. (2011) and Raposo et al. (2011). Biological methane production (BMP) tests were performed in 1 L custom-made tubular reactors and lasted 28 d. Every reactor was filled

with the material at inoculum to substrate (ISR) ratios (VS basis) that ranged from 1.85 to 3.27 (Table 2) and at an approximately 90% (wb) initial moisture content. ISR ≥ 2 has never been reported as inhibitory in AD and has been also suggested as a mandatory ratio for future standardized AD tests in batch mode (Raposo et al., 2011). Biogas pressure was measured by a digital manometer (Model SMC ZSE30, Japan). Biogas composition was measured via gas chromatography, as described in Ponsá et al. (2010), to calculate methane generation rates. All treatments were prepared in triplicates and incubated at a controlled temperature of 37°C.

The cumulative production of biogas and methane was calculated by fitting the modified Gompertz model (Eq. 2) to the experimental cumulative methane production curves. The SigmaPlot® 12.0 software (Systat Software Inc., California, USA) was used to obtain the equation parameters, namely P, R_{max} and λ (Ponsá et al., 2010).

$$M = P * exp\left\{- exp\left[\frac{R_{max} * e}{P}\ (\lambda - t) + 1\right]\right\} \qquad \text{(Eq. 2)}$$

Where: M is the cumulative BMP (l [CH_4] kg^{-1} [VS]); P is the maximum methane potential (l [CH_4] kg^{-1} [VS]); t is the time (day); R_{max} is the maximum methane production rate (l [CH_4] kg^{-1} [VS] day^{-1}) and λ the lag phase (day).

Results of BMP experiments were expressed per mass of VS of the substrate. For the digestion experiments in which pretreated OFMSW was added (in the solid form or as liquid extract), the result of the BMP test was expressed per mass of VS of simulated OFMSW. Anaerobic control runs with the pretreated material were performed as well to

allow the calculation of the net BMP of the simulated OFMSW after subtracting the biogas produced by that pretreated material.

All yields were expressed per mass of VS of simulated OFMSW. That is, from the gross methane production of the mixture, the corresponding methane productions of the other additives (inoculum, extract) were subtracted so that to finally calculate the net methane production of the OFMSW only.

2.3. Analytical Techniques

2.3.1. Basic characterization of substrates

Dry matter (DM), total organic matter (OM) and pH were determined according to the standard procedures following the Test Methods for the Examination of Composting and Compost (Puyuelo et al., 2011).

2.3.2. Enzymatic activity determinations during the aerobic experiments

The amylase enzyme activity was quantified through the release of reducing sugars using starch as substrate in 50 mM citrate buffer at a concentration of 0.5% as described in Omemu et al. (2005). 800 μL of corn starch and 200 μL of enzymatic extract were incubated at 60 °C for 1h. The protease activity was determined using a modified method described by Alef & Nannipieri (1995). One mL aliquot of enzyme extract was added to 5 mL of casein solution at 2% and was incubated at 50 °C under stirring for 2 h. Furthermore, enzymatic activity was reported as (U g^{-1} DM), where one unit (U) is the amount of enzyme

that in an enzymatic reaction catalyzes the conversion of 1 μmol of substrate per minute. All analyses were performed at least in duplicate.

2.3.3. Reducing sugars, proteins and fatty acids measurements during the aerobic experiments

Reducing sugars were determined according to a classic method previously described (Miller et al., 1960). The results were expressed as mg reducing sugar per mg of DM. Soluble protein was measured according to the method proposed by Gerhardt et al. (1994) and reported as a mg of protein per mg of DM. Fatty acids quantification was determined by extracting a 400 mg sample in 3 mL of n-heptane that were stirred in the vortex mixer for 30 seconds. The extracts were centrifuged (9.800 xg, 10 min, 4 °C), then 2.5 mL of the recovered organic phase was mixed with 0.5 mL of copper pyridine acetate solution (50 g L^{-1}, pH 6.1) and a final stirring of 30s. The absorbance at 715 nm was measured and a calibration curve was constructed using oleic acid concentrations of 0-10 mM (Hernández-Rodríguez et al., 2009). Fatty acids quantification was reported as mM per g of DM.

2.4. Statistical analysis

All measurements and tests were carried out in triplicate and the results were expressed as means ± standard deviation. Tukey's HSD test was used to compare the means and to reveal significant differences among samples (at $\alpha = 0.05$). Statistical analysis was performed using the package Sigma Plot (Systat Software Inc., San Jose, Cal.).

3. Results and Discussion

3.1. Aerobic degradation process as a pre-treatment of AD experiments

3.1.1. Evolution of the degradation aerobic process

The temperature profile of triplicates during the process is shown in Figure 1A. The profile is typical for aerobic degradation processes. A maximum of 72ºC was observed on day 5. Initial moisture of OFMSW (Table 1) was in an appropriate range for the growth of microorganisms and was kept observed to be kept constant during the process (around 60%).

Oxygen uptake rate and accumulated oxygen during the aerobic degradation of raw OFMSW is shown in Figure 1B and C respectively. The OUR showed an initial peak presumably as a consequence of the presence of readily biodegradable compounds at the beginning of the process (Martínez-Valdez et al., 2015). A pronounced decrease was observed later until the maximum OUR was achieved at around day 5 (3.1 ± 0.4 g O_2 kg^{-1} DM h^{-1}). The maximum OUR and the peak of temperature were achieved at the same time, as is common in composting experiments (Puyuelo et al., 2010). The accumulated oxygen profile showed a short lag phase for all the replicates, with a final value between 363.5 and 491.1 g O_2 kg^{-1} DM h^{-1} after 11 days of composting.

The pH decreased slightly at the beginning of the process, thereafter increased progressively until 8.8 on day 5 and remained like this until the end of the process (Figure 2A). This behavior during the process of aerobic degradation has been described as a consequence of the high initial concentration of organic acids produced during the aerobic

degradation of the OFMSW. Thereafter, an increase in pH due to mineralization of organic compounds and ammonia production was observed (Eklind & Kirchmann, 2000).

Reducing sugars observed a maximum at around day 4 and remained constant after day 7. It is important to note that the initial concentration in OFMSW was higher than in the pretreated waste (Table 1). This is expected, since, during the aerobic stage, carbohydrate monomers, proteins and lipid monomers are consumed, since they are soluble and readily biodegradable. Amylase activity (Figure 2C), which initially had an activity of 2.8 U g^{-1} DM, decreased after day 1 to 0.2 U g^{-1} DM and, thereafter, increased progressively up to 2 U g^{-1} DM on day 6.

The proteolytic activity (Figure 2E) showed a first peak on day 5 reaching 16.4 U g^{-1} DM. However, an additional increase was observed after day 8 reaching values up to 25.4 U g^{-1} DM. This was probably due that proteases taking part in nitrogen mineralization by degrading low molecular weight proteins (Vargas-García et al., 2010). Free fatty acids also peaked on days 4-6 reaching concentrations up to 400 mM g^{-1} DM (Figure 2F). The increase in the concentration of soluble protein (Figure 2D) and free fatty acids can be related to the increase of metabolic activity during the aerobic degradation of OFMSW (Tejada et al., 2009).

Therefore, it can be observed that the maximum concentration of reducing sugars and the enzymatic activities are in agreement with the maximum OUR achieved. Maximum enzymatic activity has been related to the maximum metabolic activity, which can be measured indirectly through the oxygen uptake rates (Puyuelo et al., 2010; Saucedo-Castañeda et al., 1994).

3.1.2. Selection of the optimal time to aerobically pre-treat OFMSW before its use in the anaerobic digestion experiments

As commented, a peak of metabolic activity was reached on the 5th day based on most of the parameters recorded (OUR and enzymatic activities). Those maximum values and the days that they were reached are summarized in Table 3. Also an important change in the pH of OFMSW was observed at day 5. At the beginning, pH was acidic (4.74), but after pretreatment an increase in pH in the range of 6.35 to 7.29 was achieved, approaching optimal values for AD. Also, there was not a significant loss in the VS during the aerobic pre-treatment (Table 1), suggesting that the potential to produce biogas can remain still high.

It is expected that the readily degradable organics will be practically removed after 5 days of aerobic degradation process. This fact can positively affect the anaerobic digestion process, since the acid generation during the AD process, commonly attributed to the presence of readily degradable organics, will be limited.

As a conclusion the 5th day of aerobic degradation of OFMSW was selected as the appropriate time to remove material from the aerobic process and to use it in the anaerobic digestion process of S-OFMSW as a co-substrate. In addition to the solid, liquid extracts where obtained from that 5-day aerobically pretreated OFMSW for use in the AD experiments. The addition of specific enzymes such as carbohydrase protease and lipase are expected to enhance the hydrolysis step in anaerobic digestion as previously reported (Kiran et al., 2015; Lim & Wang, 2013). Based on that notion, Kiran et al. (2015) had

applied enzymatic pretreatment of food waste with a fungal mash rich in hydrolytic enzymes that was produced by solid-state fermentation; this enzymatic addition resulted in 2.3 to 3.5 times higher biomethane yield and production rates compared to those without pretreatment. This was due to the hydrolysis and breakdown of lignocellulosic material in the aerobic pretreatment step that allowed the faster hydrolysis, and thus faster decomposition of wastes in the subsequent anaerobic step, without removing much of the biodegradable carbon. Lim and Wang (2013) reported that the aerobic pretreatment step resulted in a greater VFA formation due to the enhanced activities of the hydrolytic and acidogenic bacteria.

3.2. Anaerobic digestion experiments

3.2.1. Methane potential of the OFMSW

Methane production during anaerobic batch test of the different assays, calculated as explained in section 2.2.2, is shown in Figure 3. The parameters obtained after fitting the methane production experimental data to the Gompertz model (Eq. 2), methane potential (P), the maximum rate of methane production (R_{max}) and the lag time (λ) are shown in Table 4.

It is important to highlight that S-OFMSW had a maximum methane potential of 507 NL_{CH4} kg^{-1} VS and OFMSW (used in aerobic degradation experiments) of 518 NL_{CH4} kg^{-1} VS, being both statistically similar (at $p<0.05$) as shown in Table 4. This result validates the use of S-OFMSW in the AD assays, indicating that the simulation of OFMSW was close to reality. On the other hand, the R_{max} of the S-OFMSW and OFMSW were statistically different at $p<0.05$, being 75 and 54 $NLCH_4$ kg^{-1} VS d^{-1} respectively. This

difference can be attributed to the potential presence of slowly biodegradable matter in raw OFMSW that, as commented, was collected already in a mixture with bulking agent that have a high lignocellulosic content.

3.2.2. Methane potential after co-substrate addition

According to Table 4, if S-OFMSW is co-digested with the solid aerobically pre-treated OFMSW (50%), the maximum methane potential (expressed per kg VS of S-OFMSW basis) is increased by 19% (see Table 4). No such significant increase was, however, observed when the extract from the aerobically pretreated OFMSW was used as co-substrate. It is reminded that the aim to use liquid extract from pre-treated OFMSW was to use it as an enzymatic cocktail in digestion experiments replacing the solid material. Since extracellular enzymes carry out the hydrolysis, some authors have investigated the direct addition of hydrolytic enzyme to enhance this stage for anaerobic digestion. Pleissner et al. (2014) and Kim et al. (2006) indicated that there was enhancements in the hydrolysis step due to the addition of the enzymes during anaerobic digestion. In addition, Gerassimidou et al. (2013) showed that a short term (8 day) aerobic pretreatment of OFMSW increased biogas potential compared to untreated OFMSW and led to a faster establishment of the methanogenic phase. However, when this kind of pretreatment is realized, the selection of appropriate enzymes and sizeable enzyme activity is fundamental to achieve significant results (Cesaro & Belgiorno, 2014; Kondusamy & Kalamdhad, 2014).

Although no improvement was observed after adding the liquid extract, the increase of the maximum production rate (Rmax) by almost 40% after the use of the pretreated solid clearly indicated an improvement of the hydrolytic phase. Accordingly, an incomplete enzyme extraction from the pre-treated solid can explain the fact that this improvement was not observed when the extract was used directly (see Table 4). Also, this improvement could be due to a synergistic effect observed in an anaerobic co-digestion process (Mata-Avarez et al., 2014). Sawatdeenarunat et al. (2015) stated that the co-digestion of carbohydrate-rich lignocellulosic biomass with other waste has significant implications in balancing the C/N ratio. The establishment and maintenance of an appropriate C/N ratio was one of the key factors surrounding a successful co-digestion. Vasmara et al. (2015) found a positive correlation between CH_4 accumulation daily rate and straw enzymatic digestibility. In co-digestion with pig slurry, straw pre-treated with *Ceriporiopsis subvermispora* for 10 weeks, showed an accumulation daily rate of 17.4 mL d^{-1} g^{-1} VS, significantly higher (17%) than that of the control. In addition, the time to reach the maximum CH_4 production was shortened on average from 34 to 21 days in co-digestion with pig slurry, in comparison with pre-treated mono-digested wheat straw.

3.3. Combination of aerobic and anaerobic treatment for OFMSW

As commented in the introduction, despite the benefits of aerobic pre-treatment prior to anaerobic digestion, one has to consider the potential loss of biogas yield due to the loss of organic carbon during the aerobic pretreatment step. Due to the aerobic degradation, the biogas production of the pre-treated OFMSW was 18% lower than that generated with raw OFMSW. It could be an important loss of biogas potential, but it is important to note that only one fraction of the OFMSW will be pre-treated before AD. So, when the

anaerobic co-digestion was performed using S-OFMSW with pre-treated OFMSW in a ratio 1:1 (w/w), there was an increase in the methane potential of the S-OFMSW only by approximately 20% in both the real data and the model estimates, compared to the S-OFMSW alone, and the maximum methane production rate also increased by 39%. This is probably attributed to reasons related to co-digestion effects, such as the improvement of the balance of nutrients and the positive synergisms established in the digestion medium (Mata-Alvarez et al., 2014; Abudi et al., 2016; Yong et al., 2015). In these sense, it seems to be a good a compromise to direct one part of the OFMSW to aerobic pre-treatment for later use in the co-digestion of raw OFMSW. Thus, the combination of aerobic and anaerobic treatments could be an effective mode to apply the benefits of a short aerobic pre-treatment. More experiments will be required to optimize the ratio in the co-digestion of aerobically pre-treatment OFMSW versus OFMSW.

4. Conclusions

- The duration of 5 days was found to be the optimum time to aerobically pretreat the OFMSW prior to co-digesting it with raw OFMSW. At that time, the maximum enzymatic activity and maximum oxygen uptake rate were recorded.
- The addition of 5-day aerobically pretreated OFMSW in solid form to raw OFMSW resulted in a 20% net increase of the methane production compared to raw OFMSW. The R_{max} was also increased by 39% while no significant differences in the lag time of the methanogenic phase were observed.
- On the other hand, the addition of the liquid extract that was obtained from the 5-day pretreated OFMSW did not result in a statistically significant increase of the net methane production of the raw OFMSW.

Conclusively, it appears that it is better to mix pretreated solid waste with raw OFMSW rather than mixing the liquid extract obtained from those pretreated waste. This, however, inevitably leads to the use of larger digesters compared to if only the liquid extract had been used.

Acknowledgements

Authors thank the financial support provided by the Spanish Ministerio de Economía y Competitividad (Project CTM2015-69513-R). F.J. Martínez-Valdez is grateful to CONACyT, Mexico, for a PhD scholarships (no.237051). Dimitrios Komilis thanks TECNIOspring programme for the 2014-2016 incoming fellowship in UAB (no. TECSPR13-1-0006). Raquel Barrena thanks TECNIOspring programme for the outgoing + return fellowship (no. TECSPR15-1-0051).

References

Abudi, Z.N., Hu, Z., Sun, N., Xiao, B., Rajaa, N., Liu, C., Guo, D. 2016. Batch anaerobic co-digestion of OFMSW (organic fraction of municipal solid waste), TWAS (thickened waste activated sludge) and RS (rice straw): Influence of TWAS and RS pretreatment and mixing ratio. Energy, 107, 131-140.

Agència de Residus de Catalunya, 2006. Program for the Management of Municipal Solid Wastes in Catalonia, PROGREMIC, 2007–2012 (in Catalan)

Alef, K., Nannipieri, P. 1995. Methods in applied soil microbiology and biochemistry. Academic press.

Álvarez, J., Otero, L., Lema, J. 2010. A methodology for optimising feed composition for anaerobic co-digestion of agro-industrial wastes. *Bioresource Technology*, **101**(4), 1153-1158.

Ariunbaatar, J., Panico, A., Esposito, G., Pirozzi, F., Lens, P.N. 2014. Pretreatment methods to enhance anaerobic digestion of organic solid waste. Applied energy, 123, 143-156.

Brummeler, E.t., Koster, I.W. 1990. Enhancement of dry anaerobic batch digestion of the organic fraction of municipal solid waste by an aerobic pretreatment step. Biological Wastes, 31(3), 199-210.

Cesaro, A., Belgiorno, V. 2014. Pretreatment methods to improve anaerobic biodegradability of organic municipal solid waste fractions. Chemical Engineering Journal, 240, 24-37.

Eklind, Y., Kirchmann, H. 2000. Composting and storage of organic household waste with different litter amendments. II: nitrogen turnover and losses. Bioresource Technology, 74(2), 125-133.

Gerhardt, P., Murray, R., Wood, W.A., Krieg, N.R. 1994. Methods for general and molecular bacteriology. American Society for Microbiology Washington, DC.

Güelfo, L.F.-., Álvarez-Gallego, C., Sales, D., Romero, L. 2011. The use of thermochemical and biological pretreatments to enhance organic matter hydrolysis and solubilization from organic fraction of municipal solid waste (OFMSW). Chemical Engineering Journal, 168(1), 249-254.

Hernández-Rodríguez, B., Córdova, J., Bárzana, E., Favela-Torres, E. 2009. Effects of organic solvents on activity and stability of lipases produced by thermotolerant fungi in solid-state fermentation. Journal of Molecular Catalysis B: Enzymatic, 61(3–4), 136-142.

Kim, H.J., Kim, S.H., Choi, Y.G., Kim, G.D., Chung, T.H. 2006. Effect of enzymatic pretreatment on acid fermentation of food waste. Journal of Chemical Technology and Biotechnology, 81(6), 974-980.

Kiran, E.U., Trzcinski, A.P., Liu, Y. 2015. Enhancing the hydrolysis and methane production potential of mixed food waste by an effective enzymatic pretreatment. Bioresource Technology, 183, 47-52.

Kondusamy, D., Kalamdhad, A.S. 2014. Pre-treatment and anaerobic digestion of food waste for high rate methane production–A review. Journal of Environmental Chemical Engineering, 2(3), 1821-1830.

Lim, J.W., Wang, J.-Y. 2013. Enhanced hydrolysis and methane yield by applying microaeration pretreatment to the anaerobic co-digestion of brown water and food waste. Waste Management, 33(4), 813-819.

Martínez-Valdez, F., Martínez-Ramírez, C., Martínez-Montiel, L., Favela-Torres, E., Soto-Cruz, N., Ramírez-Vives, F., Saucedo-Castañeda, G. 2015. Rapid mineralisation of the Organic Fraction of Municipal Solid Waste. Bioresource Technology, 180, 112-118.

Mata-Alvarez, J., Dosta, J., Romero-Güiza, M., Fonoll, X., Peces, M., Astals, S. 2014. A critical review on anaerobic co-digestion achievements between 2010 and 2013. Renewable and Sustainable Energy Reviews, 36, 412-427.

Mata-Alvarez, J., Mace, S., Llabres, P. 2000. Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. Bioresource Technology, 74(1), 3-16.

Miller, G.L., Blum, R., Glennon, W.E., Burton, A.L. 1960. Measurement of carboxymethylcellulase activity. Analytical Biochemistry, 1(2), 127-132.

Omemu, A., Akpan, I., Bankole, M., Teniola, O. 2005. Hydrolysis of raw tuber starches by amylase of Aspergillus niger AM07 isolated from the soil.

Pleissner, D., Kwan, T.H., Lin, C.S.K. 2014. Fungal hydrolysis in submerged fermentation for food waste treatment and fermentation feedstock preparation. Bioresource Technology, 158, 48-54.

Ponsá, S., Gea, T., Sánchez, A. 2011. Anaerobic co-digestion of the organic fraction of municipal solid waste with several pure organic co-substrates. Biosystems Engineering, 108, 352-360.

Puyuelo, B., Gea, T., Sánchez, A. 2010. A new control strategy for the composting process based on the oxygen uptake rate. Chemical Engineering Journal, 165, 161-169.

Raposo, F., De la Rubia, M.A., Fernández-Cegrí, V., Borja, R., 2011. Anaerobic digestion of solid organic substrates in batch mode: an overview relating to methane yields and experimental procedures. Renewable and Sustainable Energy Reviews, 16, 861-877.

Saucedo-Castañeda, G., Trejo-Hernández, M.R., Lonsane, B.K., Navarro, J.M., Roussos, S., Dufour, D., Raimbault, M. 1994. On-line automated monitoring and control systems for CO2 and O2 in aerobic and anaerobic solid-state fermentations. Process Biochemistry, 29(1), 13-24.

Sawatdeenarunat, C., Surendra, K.C., Takara, D., Oechsner, H., Khanal, S.K. 2015. Anaerobic digestion of lignocellulosic biomass: Challenges and opportunities. Bioresource Technology, 178, 178-186.

Tejada, M., García-Martínez, A.M., Parrado, J. 2009. Relationships between biological and chemical parameters on the composting of a municipal solid waste. Bioresource Technology, 100(17), 4062-4065.

Tian, H., Duan, N., Lin, C., Li, X., Zhong, M. 2015. Anaerobic co-digestion of kitchen waste and pig manure with different mixing ratios. Journal of Bioscience and Bioengineering, 120(1), 51-57.

Vargas-García, M., Suárez-Estrella, F., López, M., Moreno, J. 2010. Microbial population dynamics and enzyme activities in composting processes with different starting materials. Waste Management, 30(5), 771-778.

Vasmara, C., Cianchetta, S., Marchetti, R., Galletti, S. 2015. Biogas production from wheat straw pre-treated with ligninolytic fungi and co-digestion with pig slurry. Environ. Eng. Manag. J, 14, 1751-1760.

Yong, Z., Dong, Y., Zhang, X., Tan, T. 2015. Anaerobic co-digestion of food waste and straw for biogas production. Renewable Energy, 78, 527-530.

Table 1. Characterization of materials used in the experiments

Biological Material	pH	Moisture (% wb)	VS (% db)	Reducing sugars (mg g^{-1} DM)
OFMSW	5.61	61 ± 2^{d}	74 ± 1^{b}	55.2 ± 2.4^{c}
S-OFMSW	4.74	79 ± 2^{c}	97 ± 1^{a}	90.8 ± 0.6^{a}
Pretreated OFMSW in solid form after 5d	6.35	51 ± 3^{d}	73 ± 4^{b}	8.0 ± 0.3^{d}
Liquid extract of pre-treated OFMSW after 5d	6.35	96 ± 0.03^{a}	72 ± 0.4^{b}	26.0 ± 1.3^{b}
Inoculum	7.29	89 ± 0.5^{b}	59 ± 0.4^{c}	n.d

db: dry basis; wb: wet basis; VS: volatile solids; n.d: not determined, DM: dry matter, OFMSW: Organic fraction of municipal solid waste, S-OFMSW: Simulated OFMSW. Different letters indicate statistically different means at $p < 0.05$.

Table 2. Experimental design of anaerobic experiments

	Substrates and mixtures	ISR (g $VS_{inoculum}$/g $VS_{substrate}$)
Control AD experiments	S-OFMSW	3.27
	Aerobically pre-treated OFMSW after 5 days (solid)	1.85
	Aerobically pretreated OFMSW after 5 days (liquid extract)	5.91
AD experiments	S-OFMSW + aerobically pre-treated OFMSW after 5 days (solid)	2.37
	S-OFMSW + aerobically pre-treated OFMSW after 5 days (liquid extract)	2.11

All runs were performed in triplicate; ISR: Inoculum to substrate ratio (on a VS basis).

Table 3. Maximum values of all parameters obtained during the aerobic degradation experiments.

Parameter	Units	Maximum value reached	Day
OUR	mg O_2 g^{-1} DM h^{-1}	3.1 ± 0.4	5
Cumulative oxygen uptake	mg O_2 g^{-1} DM	427 ± 64	-
Temperature	°C	72 ± 1	5
Reducing sugar	mg g^{-1} DM	31.2 ± 4.8	4
Amylase activity	U g^{-1} DM	2.4 ± 0.8	6
Protease activity	U g^{-1} DM	16.4 ± 7.3	From day 5
Protein concentration	mg g^{-1} DM	3.3 ± 0.3	5
Fatty acid concentration	mMol g^{-1} DM	395 ± 73	6

Table 4. Actual methane production (P_{real}) after 30 d and calculated maximum methane potential (P), maximum methane production rate (R_{max}) and lag phase (λ) estimated by the fitting of the Gompertz model to the data.

Test	**P_{real} (NL CH_4 kg^{-1} VS of S-OFMSW)**	**P_{model} (NL CH_4 kg^{-1} VS of S-OFMSW)**	**R_{max} (NL CH_4 kg^{-1} VS of S-OFMSW day^{-1})**	**λ (day)**
Control AD experiments				
S-OFMSW	518 ± 1.5^{b}	510 ± 2^{b}	74 ± 3^{b}	0.65 ± 0.11^{a}
OFMSW	524 ± 30^{b}	522 ± 27^{b}	53 ± 1^{c}	0.86 ± 0.12^{a}
Pre-treated OFMSW	430 ± 33^{c}	413 ± 35^{c}	42 ± 6^{d}	1.05 ± 0.3^{a}
AD experiments				
S-OFMSW + aerobically pre-treated OFMSW after 5 days *(liquid extract)*	568 ± 17^{b}	538 ± 14^{b}	55 ± 2^{c}	0.2 ± 0.41^{a}
S-OFMSW + aerobically pre-treated OFMSW after 5 days *(solid material)*	620 ± 16^{a}	602 ± 14^{a}	103 ± 4^{a}	0.54 ± 0.14^{a}

Different letters indicate statistically different means at $p < 0.05$. For the OFMSW control experiment, the results are expressed in NLCH_4 kg^{-1} VS of OFMSW.

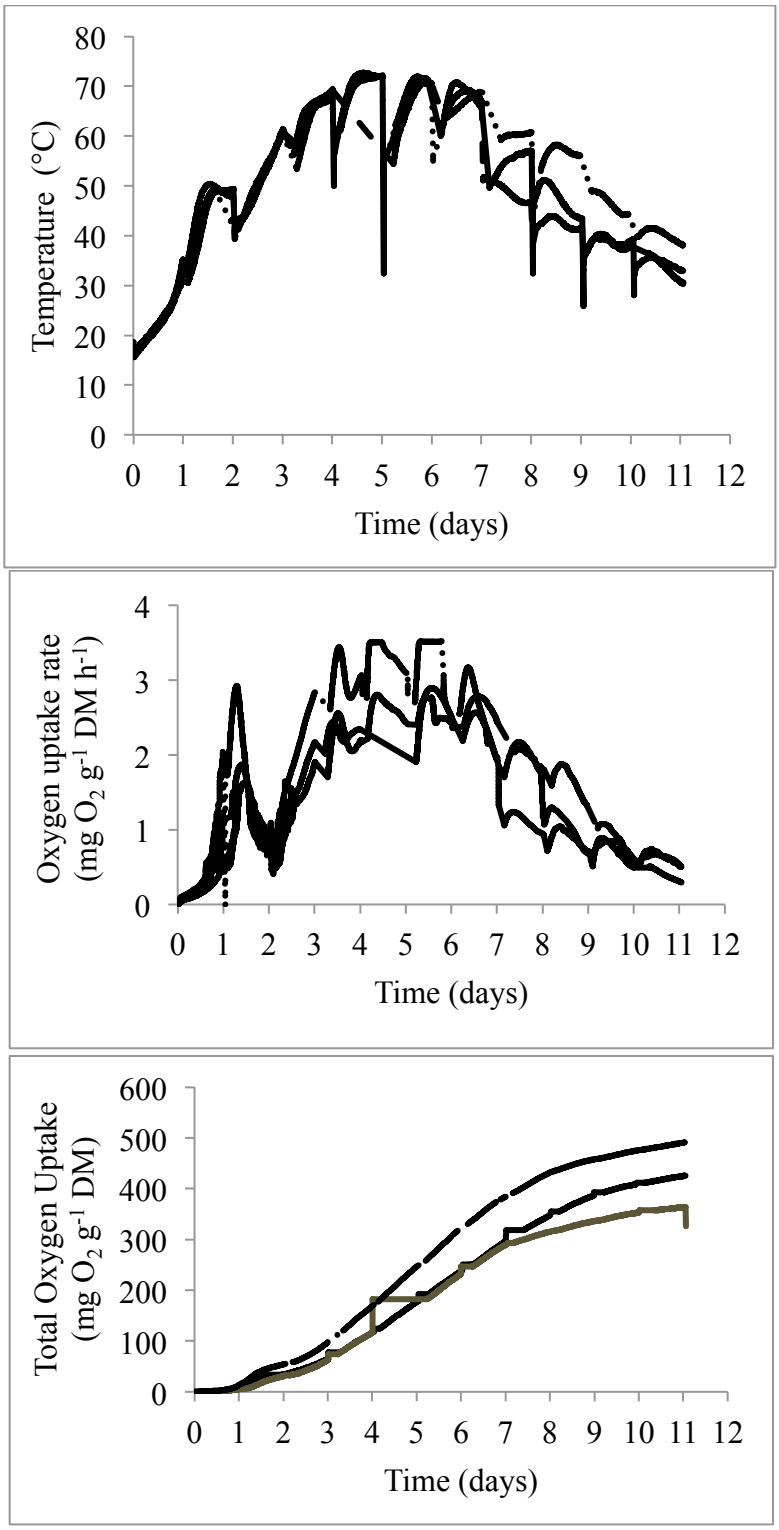

Figure 1. Evolution of aerobic biodegradation process of the raw OFMSW in a 10 L reactor (results are from triplicates); top: temperature profile; middle: oxygen uptake rate (OUR) profile and bottom: cumulative oxygen uptake.

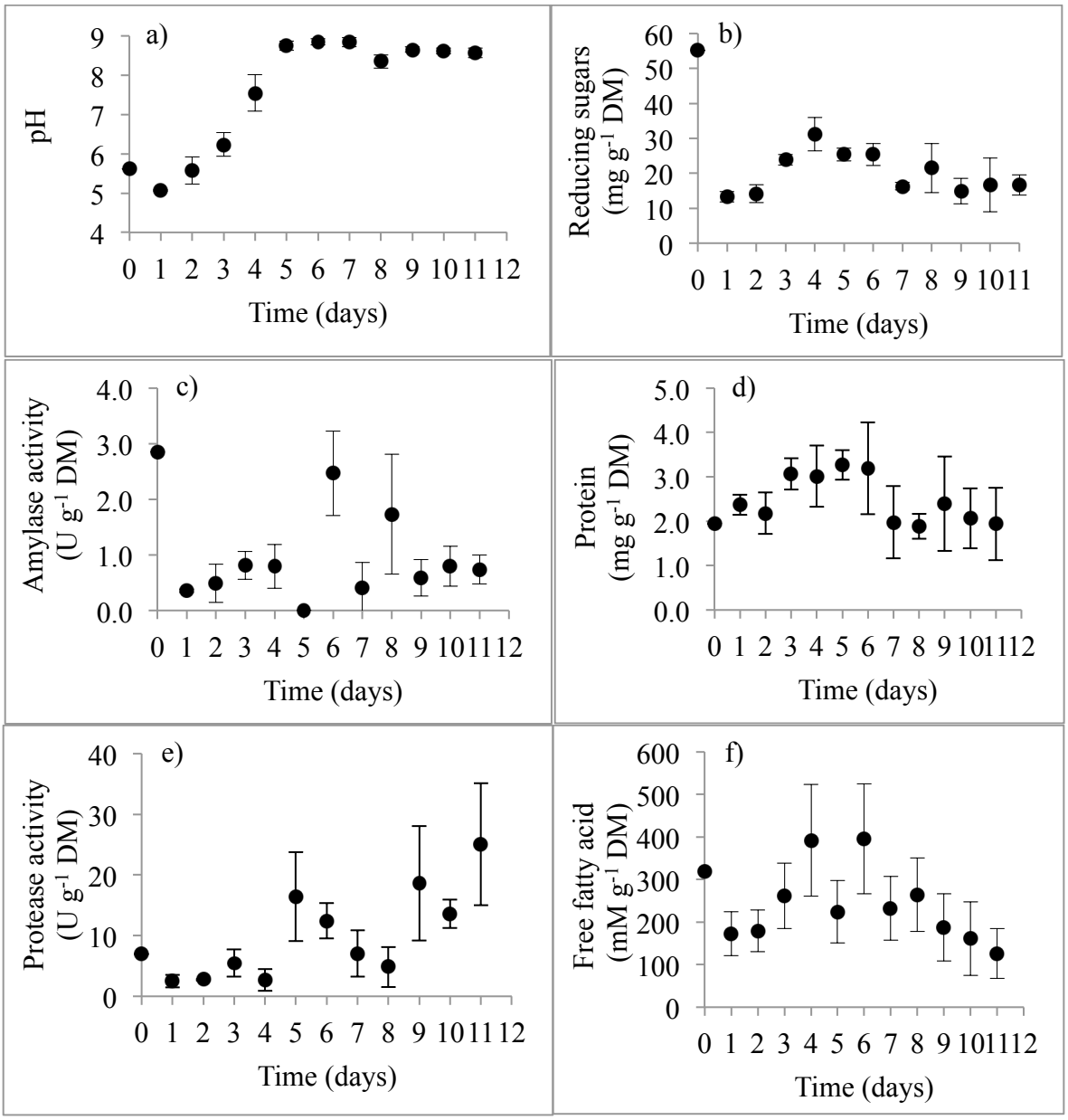

Figure 2. Evolution of different parameters during the aerobic degradation of OFMSW a) pH; b) reducing sugars; c) amylase activity; d) total protein, e) protease activity and f) free fatty acids. Error bars represent the standard deviation of triplicates.

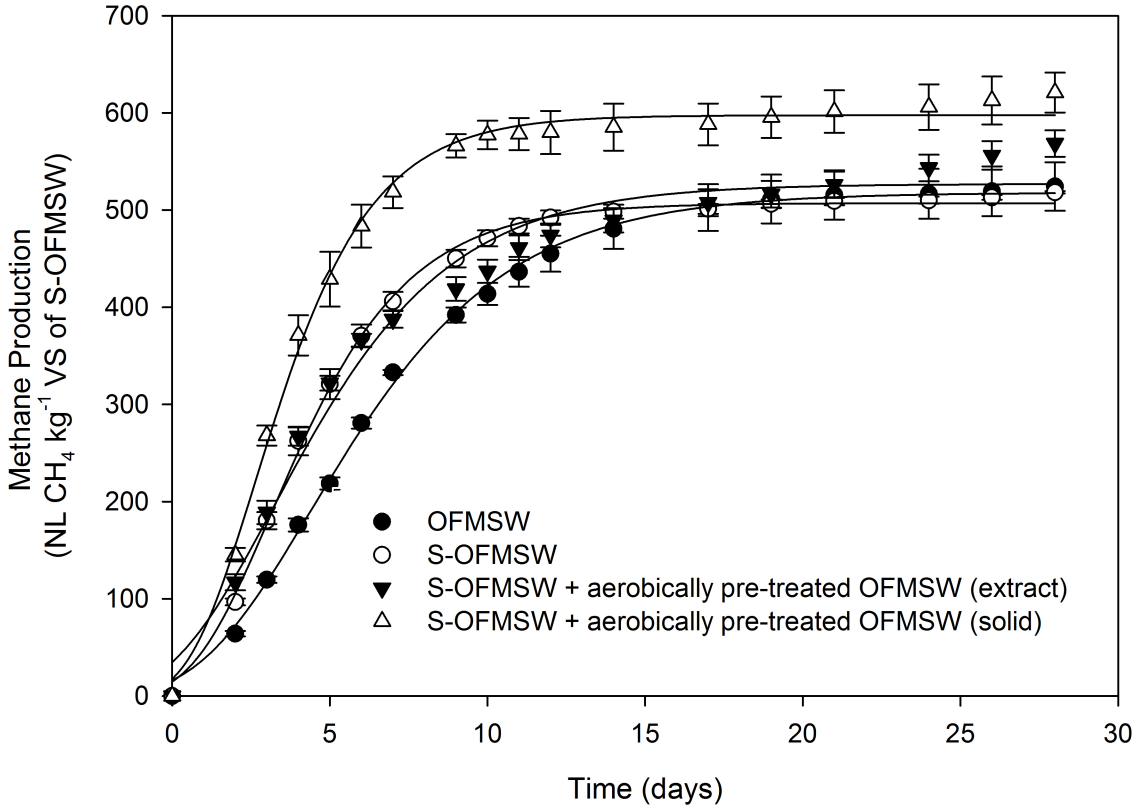

Figure 3. Net cumulative methane production during the anaerobic digestion experiments (results are expressed per mass of VS of S-OFMSW or OFMSW included in the mixture). The solid curved line represents the Gompertz model fit.