Assessment of two-phase olive mill solid waste and microalgae co-digestion to improve methane production and process kinetics

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Abstract

Olive mill solid waste (OMSW) is a pollutant waste coming from olive oil elaboration by the two-phase centrifugation system. OMSW has a high organic matter content and unbalanced carbon to nitrogen (C/N) ratio, 31/1, which avoids obtaining high methane yields in the anaerobic digestion of this waste. In the present study a microalgae, *Dunaliella salina*, was employed as co-substrate for the OMSW anaerobic digestion in order to decrease the C/N ratio and increase its biodegradability. Different co-digestion mixtures (C/N ratios) were studied. The increase of *D. salina* from 25% to 50% in the co-digestion mixture clearly increased the biodegradability of the sole substrates. The highest biodegradability was found for the co-digestion mixture 50% OMSW-50% *D. salina*. Nevertheless, the maximum methane production, 330 mL CH₄/g VS_{added}, and the highest methane production rate were obtained for the co-digestion mixture 75% OMSW-25% *D. salina*, keeping a C/N ratio near to 26.7/1.

Keywords: Microalgae; olive mill solid waste; co-digestion; biomethane; biochemical methane potential; kinetics.

1. INTRODUCTION

Over 2.9 million tonnes of virgin olive oil are produced annually worldwide, of which 2.4-2.6 million tonnes are produced in the European Union (IOOC 2009). Over the past decade, Spain has produced between 1.412.000 tonnes (2003/2004 season) and 1.028.000 tonnes (2008/2009 season) of olive oil, which meant 57.7% and 53% of European production (IOOC 2009). Taking into account that oil is only c.a. 20 % (w/w) of the olive, the high quantities of waste produced in the olive oil mills makes sustainable treatments necessary. 98 % of Spanish olive oil mills use the two-phase centrifugation system for olive oil extraction. The main waste produced in this system is the olive mill solid waste (OMSW). The current treatment of OMSW is based on the extraction of the residual olive oil and further combustion. This treatment is not sustainable because of the high water content of the OMSW (Azbar et al., 2004; Celma et al., 2008). There are several experimental treatments for OMSW, such as feedstock for animals, source of pharmaceutical compounds or fertilizer (Martín et al. 2003; Ramos-Cormenzana & Monteoliva-Sánchez 2000; Sierra et al. 2000). An extremely low quantity of OMSW is used in these treatments, so none could be used as an integral treatment for this problematic waste.

Anaerobic digestion of OMSW is a promising technology. Biomethane production between 200-300 mL CH₄/g COD removed and stable continuous reactor operation with organic loading rates up to 9.2 g COD/(L·d) have been already shown (Rincón et al., 2007). However, organic loading rates higher than 9.2 g COD/(L·d) resulted in a considerable process instability and inhibition. The high content in complex compounds, e. g. cellulose, hemicellulose and phenolic compounds present in the OMSW were most likely responsible for such inhibition (Rincón et al., 2007). In addition, an unbalanced carbon to nitrogen (C/N) ratio 31/1 found in OMSW most

likely also avoids a maximal biogas production from this waste.

Co-digestion is proposed in the present study as an approach to dilute complex compounds and balance the C/N ratio. The benefits of co-digestion lie in balancing the C/N ratio in the co-substrate mixture, as well as macro and micronutrients, pH, inhibitors/toxic compounds and dry matter (Hartmann & Ahring, 2005). Low levels of nitrogen, i.e. high C/N ratio, are characterized by a low pH substrate, poor buffering capacity, and the possibility of high volatile fatty acid (VFA) accumulation in the digestion process (Banks & Humphreys, 1998). Low C/N ratios contain relatively high concentrations of ammonia, exceeding that necessary for microbial growth and probably inhibiting anaerobic digestion (González-Fernandez et al., 2011; Yen & Brune, 2007). Several authors have indicated optimum C/N ratio in anaerobic digesters between 20:1 and 30:1 (Habiba et al., 2009; Yen & Brune, 2007).

Anaerobic co-digestion of organic wastes is increasingly being applied for simultaneously treatment of several agro-industrial solid wastes. Moreover, co-digestion may contribute to a more efficient use of anaerobic reactors and cost-sharing by processing different waste streams in a single equipment (Dareioti et al., 2009).

Microalgae, the common denomination for a broad group of photosynthetic prokaryotes and eukaryotes, are characterized for an efficient conversion of the solar energy to biomass. They are a promising feedstock of biomass for the production of biogas considering both their biomass as energy source and their advantages over traditional land-based energy crops (Salerno et al., 2009). However, microalgae have a very low C/N ratio, which hinders and inhibits a further anaerobic digestion. Ammonia toxicity and recalcitrant cell walls are commonly cited causes of these low methane yields (Sialve et al., 2009). Ammonia toxicity might be counteracted by co-digesting microalgae with high-carbon wastes (Salerno et al., 2009). Yen and Brune (2007)

doubled methane production of algal biomass by co-digesting it with waste paper compared with algal biomass alone, with optimum C/N ratio between 20 and 25 (Yen and Brune, 2007). It has been also reported that co-digestion of algae *Spirulina platensis* with WAS improved volatile solids reduction and dewaterability of the digestate compared to WAS alone (Yuan et al., 2012). The same authors reported that co-digestion of algae *Chlorella sp.* with WAS improved volatile solids reduction as well, however, *Chlorella sp.* had a slight negative effect on dewaterability of the digestate compared to WAS alone (Yuan et al., 2012). Algae biomass residue has also been co-digested with lipid-rich Fat, Oil, and Grease waste (FOG) to evaluate the effect on methane yield (Park and Li, 2012). Co-digestion of algae biomass residue and FOG, each at 50% of the loading, allowed for organic loading rates up to 3 g VS/(L·d), resulting in a specific methane yield of 0.54 L CH₄/(g VS·d) and a volumetric reactor productivity of 1.62 L CH₄/(L·d). Lipids were the key contributor to methane yields, accounting for 68-83% of the total produced methane (Park and Li, 2012).

Dunaliella genus is probably the most halotolerant eukaryotic organisms known, showing a remarkable degree of adaptation to a variety of salt concentrations from 0.2% to salt saturation (Kaçka, A. and Dönmez, 2008). The ability to grow at very high salt concentrations has made these microalgae an attractive candidate for industrial oil transformation which presents a high range of salinity. It could be possible to grow it up in e.g. brine table olives, reducing the need of fresh water and underlined the necessity for very low-cost culture systems. *Dunaliella salina* lacks of a rigid cell wall (Avron and Ben-Amotz, 1992) which most likely would help to the anaerobic digestion process.

The aim of this study was to investigate the possibility of improving methane yield from anaerobic digestion of OMSW in co-digestion with a specific microalga, *D. salina*, based on an optimized C/N ratio. Different co-digestion mixtures were tested in

biochemical methane potential (BMP) tests. The influence of the percentage of each co-substrate on the kinetics of the anaerobic process and ultimate methane yield were also evaluated.

2. MATERIALS AND METHODS

2.1. Two-phase olive mill solid waste

The two-phase OMSW used in the experiments was collected from the Experimental Olive Oil Mill Factory located in the ‘Instituto de la Grasa (CSIC)’, Seville (Spain). Some of the characteristics of the OMSW used in the experiments are detailed in Table 1. Before to be used, the OMSW was sieved through a 2 mm mesh for removing olive stone pieces.

2.2. Dunaliella salina

Dunaliella salina was provided as a lyophilised by Huelva University, Huelva (Spain). The main characteristics of the *D. salina* used are shown in Table 1.

2.3. Anaerobic sludge

The anaerobic sludge used as inoculum in the BMP tests was obtained from an industrial upflow anaerobic sludge blanket (UASB) reactor treating brewery wastewater in Sevilla (Spain). This inoculum was selected due to its high methanogenic activity proven in previous experiments (Rincón et al., 2013). The main characteristics of the inoculum used were: pH: 7.05, total Kjeldahl nitrogen (TKN): 0.5 ± 0.4 g TKN/kg, total solids (TS): 68.7 ± 0.7 g/kg and volatile solids (VS): 24.7 ± 1.8 g/kg.

2.4. Experimental setup

Different combinations OMSW/*D. salina* were tested: 100% OMSW; 75% OMSW-25% *D. salina*; 50% OMSW-50% *D. salina*; 25% OMSW- 75 % *D. salina* and 100% *D. salina* corresponding to C/N ratios of: 31.4, 26.7, 22.0, 17.3 and 12.6, respectively.

The biochemical methane potential (BMP) tests were carried out in a multi-batch reactor system; effective volume of reactors was 250 mL. They were continuously agitated by magnetic bars at 500 rpm and placed in a thermostatic water bath at mesophilic temperature (35 ± 2 °C).

The inoculum to substrate ratio was 2 (VS basis). For each reactor containing 239 mL of inoculum, the amount of substrate needed to give the required inoculum to substrate ratio was added together with 239 μ L of trace element solution.

The composition of the trace elements solution was: $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, 2000 mg/L; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 2000 mg/L; $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 500 mg/L; $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$, 90 mg/L; $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 50 mg/L; H_3BO_3 , 50 mg/L; ZnCl_2 , 50 mg/L; $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, 38 mg/L, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, 50 mg/L, $\text{Na}_2\text{SeO}_3 \cdot 5\text{H}_2\text{O}$ 194 mg/L and EDTA 1000 mg/L. Two reactors with inoculum and trace elements solution but without substrate addition were used as controls.

The reactors were sealed and the headspace of each flask was flushed with nitrogen at the beginning of the assay. The produced biogas was passed through 3N NaOH solution to capture CO_2 ; the remaining gas was assumed to be methane. The anaerobic digestion experiments were run for a period of c.a. 25 days until the accumulated gas production remained essentially unchanged, i.e. on the last day production was lower than 2% of the accumulated methane produced. Each experiment was carried out in duplicate.

2.5. Analytical methods

All analyses were performed according to the Standard Methods of APHA (APHA, 1998). The following parameters were measured: total chemical oxygen demand (COD), soluble chemical oxygen demand (SCOD), total solids (TS), volatile solids (VS), total alkalinity (TA), pH, Total Kjeldahl Nitrogen (TKN) and elemental C and N.

TS and VS were determined according to the standard methods 2540B and 2540E (APHA, 1998), respectively; COD was determined by the method described by Raposo et al. (2008), while SCOD was determined using the closed digestion and the colorimetric standard method 5220D (APHA, 1998). pH was analysed using a pH-meter model Crison 20 Basic. TA was determined by pH titration to 4.3 (APHA, 1998). TKN was determined using a method based on the 4500-N_{org} B of Standard Methods (APHA, 1998). C and N were determined through an Elemental Analyser LECO CHNS-932 (Leco Corporation, St Joseph, MI, EEUU).

3. RESULTS AND DISCUSSION

3.1. Influence of co-digestion on biochemical methane potential

Figure 1 shows the variation of the methane yield obtained (mL CH₄/g VS added) against digestion time (days) for the BMP tests carried out with 100% OMSW, 100% *D. salina*, and with the co-digestion mixtures 75% OMSW-25% *D. salina*, 50% OMSW-50% *D. salina*, 25% OMSW-75% *D. salina*.

The experimental methane yields observed for each co-digestion mixture (Figure 1) were compared to a calculated methane yields based on the OMSW and *D. salina* methane yields separately according to the equation 1:

$$\text{Calculated methane yield (mL CH}_4\text{/g VS}_{\text{added}}) = \% \text{ OMSW} \cdot (321) + \% D. \text{ salina} \cdot (63) \quad (1)$$

Where 321 and 63 are the experimental methane yields (mL CH₄/g VS added) obtained from 100% OMSW and 100% *D. salina*, respectively. % OMSW and % *D. salina* are the percentages of OMSW and *D. salina* in each co-digestion mixture. The experimental methane yield values obtained for all experiments performed and the calculated ones are summarized in Table 2.

Experimental BMP values were higher than the calculated methane yield from eq. 1 in each of the co-digestion mixture tested (Table 2). 28% for co-digestion mixture 75 % OMSW-25 % *D. salina*, 48% for co-digestion mixture 50 % OMSW-50 % *D. salina* and 3% for co-digestion mixture 25 % OMSW-75 % *D. salina*. According to the increase of BMP values, the biodegradability of the co-digestion mixtures were as well much higher than the biodegradability of the sole substrates (Table 2). The biomethane potential of the OMSW was found very low, as only 56.9% of the available COD is converted to methane. The biomethane potential of the *D. salina* was found very low as well, 25% of the available COD was converted to methane (Table 2). The co-digestion mixture 50 % OMSW-50 % *D. salina* had a biodegradability of 73.2 and the co-digestion mixture 75% OMSW-25% *D. salina* of 71.5. Synergy effect of the OMSW and *D. salina* co-digestion was clearly shown with these results.

Although the co-digestion mixture 50 % OMSW-50 % *D. salina* increased 48% the methane yield with respect to its calculated value and had a biodegradability of 73.2% (Table 2), the co-digestion mixture 75% OMSW-25% *D. salina* was the combination with the highest methane yield, i.e. 330 mL CH₄/g VS added. 75% OMSW-25% *D. salina* co-digestion mixture corresponded to a C/N ratio of 26.7/1, an intermediate value between 20/1 and 30/1 described as optimum range in literature (Habiba et al., 2009).

Furthermore, the methane yield value obtained for the mixture 75% OMSW-25% *D. salina* was 15.8% and 150% higher than those achieved for the mixtures 50% OMSW-50% *D. salina* and 25% OMSW-75% *D. salina*, respectively.

The unbalanced C/N ratios of the algal biomass have been reported as an important limitation factor to anaerobic digestion processes. It has been reported that the addition of waste paper (50% based on VS) in algal biomass feedstock to maintain an optimum C/N ratio (20-30) double the methane production rate (Yen and Brune, 2007). The latter authors claimed that the stimulation of the cellulose activity by the presence of the waste paper had a positive effect on the anaerobic digestion of algal cell walls (Yen and Brune, 2007). Co-digesting studies with a mixture of algae, effluent from canning industry and protein-extracted algae also demonstrated that the optimum C/N ratio to achieve a maximum methane production was found between 20 and 30 (Chen, 1987). C/N ratios lower than 20/1 lead to potential inhibition due to the presence of free ammonia whereas C/N ratios higher than 30/1 may lead to potential nitrogen limitations (Sialve et al., 2009).

The lowest methane yields obtained in the present study corresponded to the 100% *D. salina* and for the co-digestion mixture 25% OMSW-75% *D. salina*. González-Fernández et al., (2011) reported in the co-digestion of microalgal biomass constituted by *Chlorella vulgaris*, *Scenedesmus obliquus* and swine manure that the methane yield decreased from 221 to 143 mL CH₄/g COD when the percentage of total COD provided by algal biomass increased from 14.6% to 85.4%, the lowest value was achieved for the digestion of algal biomass as a sole substrate (128 mL CH₄/g COD). This result was attributed to the hemicellulosic cell wall of these two species of microalgae, which present a high resistance to bacterial degradation (González-Fernández et al., 2011).

It has been also reported the anaerobic co-digestion of cattle excreta and OMSW (Goberna et al., 2010). The mesophilic co-digestion at a 3:1 ratio rendered 1096 mL biogas/(L sludge d), value 33% higher than that of excreta alone. The methane yield resulting from the co-digestion was 179 mL CH₄/g VS added, of which 42% was attributed to OMSW (Goberna et al., 2010). This methane yield value was considerably lower than those obtained in the present work for the co-digestion mixtures 75% OMSW-25% *D. salina* (330 mL CH₄/g VS added) and 50% OMSW-50% *D. salina* (285 mL CH₄/g VS added).

3.2 Influence of co-digestion on process kinetics

3.2.1. Kinetic models of methane production

Two different periods were clearly differentiated in the evolution of methane production with time for the digestion of 100% OMSW and for the co-digestion mixtures (Figure 1).

A first stage, during the first 5 days of operation, followed by an intermediate adaptation period or lag stage, and finally, a second phase, in which the methane production rate increased gradually to become almost zero at the 15-20 days of digestion were observed (Figure 1). A similar trend was observed previously by Rincón et al (2013) with OMSW as substrate. Only the first stage was observed for the digestion of 100% *D. salina*. Therefore, OMSW digestion is clearly the reason for such a two stages methane production profile.

In order to simulate the two stages observed, two different models were selected and used as previously by Rincón et al. (2013). A first-order exponential model for the first stage which is commonly applicable to easily biodegradable substrates (Li et al.,

2012) and a second sigmoidal or logistic model for the lag and second stage with its three characteristic phases, i.e. lag, exponential increase and final stabilization step (Donoso-Bravo et al., 2010).

3.2.2. First-order exponential model

The first-order exponential model is given by the equation 2:

$$B_t = B_{max} \cdot [1 - \exp(-k \cdot t)] \quad (2)$$

where: B_t (mL CH₄/g VS_{added}) is the cumulative specific methane production, B_{max} (mL CH₄/g VS_{added}) is the ultimate methane production, k is the specific rate constant or apparent kinetic constant (days⁻¹) and t (days) is the time.

This model was applied for the first experimental stage of methane production or exponential step (first 5 days of digestion) for digestion of 100% OMSW and for co-digestion mixtures. Moreover, this model was the only one applied in the case of digestion of 100% *D. salina*.

The adjustment by non-linear regression of the pairs of experimental data (B_t , t) using the Sigmaplot software (version 11.0) allowed the calculation of the parameters k and B_{max} for this first stage of methane production (Table 3). The high values of the R² and the low values of the standard error of estimate for all cases tested demonstrate the goodness of the fit of experimental data to the model proposed for this first exponential stage (Table 3, Figure 2).

k values obtained for the first stage of digestion were very similar for digestion of 100% OMSW and for co-digestion mixtures 75% OMSW-25% *D. salina* and 50% OMSW-50% *D. salina*, with values ranging between 0.69±0.04 and 0.78±0.04 days⁻¹ (Table 3). The lowest k value, i.e. 0.49 days⁻¹, was achieved for 100% *D. salina* digestion. The low C/N ratio of the microalga alone, i.e. 12.6, is most likely the reason

for such a low k value. The highest k value in the first stage was obtained for the co-digestion mixture 25% OMSW-75% *D. salina*, i.e. 2.2 days⁻¹ (Table 3). The increase of OMSW to the co-digestion mixture resulted in lower k values but higher $B_{\max}$ than the co-digestion mixture 25% OMSW-75% *D. salina* (Table 3). The increase in the C/N ratio improved the total methane production, however, had a negative effect on the initial degradation rate. This negative effect might be attributed to an increasing concentration of complex compounds in the co-digestion mixture coming from the OMSW (Rincón et al., 2007).

3.2.3. Sigmoidal or logistic model

For the second stage of methane production, i.e. between the 5th and last day of the operating period: 24-25th day, the following logistic model (eq. 3) was used to estimate process performance (Donoso-Bravo et al., 2012; Li et al., 2012; Rincón et al., 2013):

$$B_2 = B_0 + P/[1 + \exp (-4 \cdot R_m \cdot (t - \lambda)/(P + 2))] \quad (3)$$

where: B_2 is the cumulative methane production during the second stage (mL CH₄/g VS_{added}), B_0 is the cumulative methane production at the start-up of the second stage (mL CH₄/g VS_{added}) and should approximately coincide with the value of $B_{\max}$ obtained at the end of the first stage, P is the maximum methane production obtained in the second stage (mL CH₄/g VS_{added}), R_m is the maximum methane production rate (mL CH₄/(g VS_{added} · d)) and λ is the lag time (days).

The logistic model assumes the rate of methane production to be proportional to microbial activity (Altas, 2009). The logistic model fairly fits the methane production during the second stage (5-25 days): an initial lag period followed by an exponential increase and a final stabilization at a maximal production level (Figure 2, Table 4).

This model has been previously used for estimating the methane production in

batch anaerobic digestion experiments of different substrates such as landfill leachate, herbaceous grass materials, sewage sludge, etc. (Altas, 2009; Donoso-Bravo et al., 2010; Li et al., 2012; Pommier et al., 2006).

Table 4 summarizes the kinetic parameters obtained from the logistic model in the BMP tests of the different mixtures tested. The highest estimated lag periods for the logistic model were found for the co-digestion mixtures 25% OMSW-75% *D. salina* and 50% OMSW-50% *D. salina*. The increase of *D. salina* in the co-digestion mixture clearly increased the lag period for the second stage. The high estimated lag time found for mixtures containing the highest proportion of microalgae may be attributed to the high protein content of the algal biomass, which leads to a high ammonium release, thus inhibiting the anaerobic microorganisms (Sialve et al., 2009). The lowest estimated lag period corresponded to the mixture 75% OMSW-25% *D. salina*, i.e. 6.4 days (Table 4). The estimated lag period for 100% OMSW was 9.9 days (Table 4). This observation indicates that, although the increase of *D. salina* over 50% promotes inhibition to the microbial community, an adequate addition would definitely increase the methane production.

The value of R_m for the mixture 75% OMSW-25% *D. salina* was 60% and 155% higher than those obtained for the mixtures 50% OMSW-50% *D. salina* and 25% OMSW-75% *D. salina* respectively. To be specific it was observed a decrease in the maximum methane production rate (R_m) of this stage from 48.1 to 18.8 mL CH₄/(g VS·d) when the percentage of *D. salina* in the mixture increased from 25% to 75%.

The ammonia release during the co-digestion of increased concentrations of microalgae could explain the poorer digestion performance and slower kinetics when the percentages of microalgae in the co-digestion mixture were increasing (González-Fernández et al., 2011; Sialve et al. 2009).

The first derived of B_2 with respect to the digestion time gives the evolution of the methane production rate, which maximum corresponds to R_m , with time during the second stage (Figure 3). The degradation rate of the co-digestion mixture 75% OMSW-25% *D. salina* was the fastest of the three conditions tested, achieving the R_m , i.e. 48.1 mL CH₄/(g VS·day), after 6.3 days of digestion time. The time to achieve R_m was much higher for the other tests than for the 75% OMSW-25% *D. salina*. 50% OMSW-50% *D. salina* achieved R_m , i.e. 30.1 mL CH₄/(g VS·day), after 12.5 days of digestion. 25% OMSW-75% *D. salina* achieved R_m , i.e. 18.8 mL CH₄/(g VS·day), after 12.5 days of digestion as well. 100% OMSW achieved R_m , i.e. 38.4 mL CH₄/(g VS·day), after 9.4 days. The co-digestion mixture 75% OMSW-25% *D. salina* was the fastest and the one that produces the highest amount of biomethane among all the co-digestion mixtures tested.

3.3. Influence of co-digestion on the olive mill sustainability

The use of *D. salina*, together with OMSW (75% OMSW-25% *D. salina*) allows obtaining higher methane yields from OMSW than using OMSW alone. The energy obtained in the digestion process could be used to keep the mesophilic operating temperature (35 °C) of the anaerobic reactor and even in the own olive oil elaboration. Moreover, the effluents obtained in the anaerobic digester might be used as fertilizer in olive trees fields and as nutrient source for new microalgae cultivation. All this improves the whole sustainability of the olive oil elaboration system by means of close loops. Furthermore, the use of a saline microalga as *D. salina* would allow the use of sea water or salted concentrated industrial streams, e.g. olive brine, as growth media, decreasing the need of fresh water.

4. CONCLUSIONS

Anaerobic co-digestion of OMSW and *D. salina* with a mixture of 75%-25% respectively, keeping a C/N ratio of 26.7, increased the methane yield and the methane production rate compared to anaerobic digestion of 100% OMSW, 100% *D. salina* and other co-digestion mixture percentages. Nevertheless, anaerobic co-digestion of 50% OMSW-50% *D. salina* had a higher synergic effect than the other co-digestion mixtures studied. Although the 50% co-digestion mixture increased biodegradability of OMSW and *D. salina*, the 75% OMSW-25% *D. salina* co-digestion mixture would allow operating with smaller anaerobic digesters or lower retention times and with a still high biodegradability of OMSW.

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Table 1

Characteristics of the OMSW and *Dunaliella salina* used in the experiments. Where TS: total solids, VS: volatile solids, COD: total chemical oxygen demand, SCOD: soluble chemical oxygen demand, TKN: Total Kjeldahl nitrogen, TA: total alkalinity, nd: not determined.

Parameters	Values	Values
	OMSW*	<i>D. salina</i> **
TS (g/kg)	272.2 ± 1.7	908.0 ± 7.3
VS (g/kg)	234.6 ± 2.5	435.8 ± 4.1
COD (g O ₂ /kg)	331.1 ± 0.7	272 ± 8
SCOD (g O ₂ /kg)	143.4 ± 3.2	nd
TKN (g/kg)	nd	8.4 ± 0.4
pH	4.9 ± 0.2	8.19±0.1 (1:20)***
TA (g CaCO ₃ /kg)	2.5 ± 0.0	nd

*Concentrations expressed as: weight/weight of wet sample.

Concentrations expressed as: weight/weight of lyophilised sample. * (w:v) using distillate water.

Table 2

Calculated methane yield values obtained from eq. 1, experimental data obtained through BMP test and biodegradability of the different co-digestion mixtures

C/N ratio	OMSW (%)	<i>D. salina</i> (%)	Calculated (mL CH₄/g VS added)	Experimental (mL CH₄/g VS added)	Methane yield improvement (%)	Biodegradability (COD-CH₄ / CODadded) (%)
31.4	100	0	321	321	0	56.9
26.7	75	25	257	330	28	71.5
22.0	50	50	192	285	48	73.2
17.3	25	75	128	132	3	45.8
12.6	0	100	63	63	0	24.7

Table 3

Kinetic parameters obtained from the exponential model in the BMP tests of digestion of 100% *D. salina*, 100% OMSW and for co-digestion mixtures 75% OMSW-25% *D. salina*, 50% OMSW-50% *D. salina* and 25% OMSW-75% *D. salina*,

Substrate	B_{max} (mL CH ₄ /g VS _{added})	k (days ⁻¹)	R²	S.E.E.
100% <i>D. salina</i>	62±4	0.49±0.08	0.9558	3.99
25% OMSW-75% <i>D. salina</i>	76.7±0.8	2.2±0.1	0.969	3.64
50% OMSW – 50% <i>D. salina</i>	161±3	0.69±0.04	0.958	9.72
75% OMSW – 25% <i>D. salina</i>	198±5	0.75±0.04	0.983	7.59
100% OMSW	133±2	0.78±0.04	0.991	4.26

B_{max} is the ultimate methane production, k is the specific rate constant or apparent kinetic constant. Parameters from the nonlinear regression fit: R²: coefficient of determination; S.E.E.: Standard Error of Estimate.

Table 4

Parameters obtained from the logistic model in the BMP tests of 100% OMSW and for co-digestion mixtures 75% OMSW-25% *D. salina*, 50% OMSW-50% *D. salina* and 25% OMSW-75% *D. salina*.

Substrate	B_0 (mL CH ₄ /g VS _{added})	P (mL CH ₄ /g VS _{added})	R_m (mL CH ₄ /(g VS·d))	λ (days)	R ²	S.E.E.
25% OMSW-75% <i>D. salina</i>	77.5±0.7	72.4±0.8	18.8	12.8±0.1	0.999	1.00
50% OMSW-50% <i>D. salina</i>	165±1	132±1	30.1	12.6±0.1	0.999	1.22
75% OMSW-25% <i>D. salina</i>	188±2	149±2	48.1	6.4±0.1	0.998	1.51
100% OMSW	118±10	181±11	38.4	9.9±0.2	0.995	4.98

B_0 is the cumulative methane production at the start-up of the second stage (mL CH₄/g VS_{added}), P is the maximum methane production obtained in the second stage (mL CH₄/g VS_{added}), R_m is the maximum methane production rate (mL CH₄/g VS_{added}·d) and λ is the lag time (days). R²: coefficient of determination; S.E.E.: Standard Error of Estimate.

Figure 1. Biochemical methane potential (mL CH₄/g VS added) of 100% OMSW (■), 100% *Dunaliella salina* (□) and different co-digestion mixtures tested: 75% OMSW-25% *D. salina* (◇); 50% OMSW-50% *D. salina* (▲) and 25% OMSW- 75 % *D. salina* (◆).

Figure 2. Cumulative methane yield expressed as mL CH₄/g VS added (*), first stage exponential model (---) and (---) second stage logistic model for 100% OMSW and the different co-digestion mixtures studied: 75% OMSW-25% *D. salina*, 50% OMSW-50% *D. salina*, and 25% OMSW- 75 % *D. salina*.

Figure 3. Methane production rate, expressed as mL CH₄/(gVS day) obtained during the second stage of the BMP test carried out with the 100% OMSW and the different co-digestion mixtures: 75% OMSW-25% *D. salina*, 50% OMSW-50% *D. salina*, and 25% OMSW- 75 % *D. salina*.