ORIGINAL PAPER

Enhanced Biogas Production in Pilot Digesters Treating a Mixture of Olive Mill Wastewater and Agro-industrial or Agro-livestock By-Products in Greece

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Abstract

The selection of appropriate co-substrates is very important to the feasibility of an anaerobic co-digestion process. A proper choice of co-substrate compositions leads to system balance and increased methane generation. To valorize agricultural wastes and byproducts in southern Greece, anaerobic co-digestion of four feedstocks (olive mill wastewater—OMW, poultry manure—PM, liquid pig manure—LPM, and cheese whey—CW) was studied to produce biogas for renewable energy generation. Pilot- scale continuous co-digestion approaches were adopted to carry out the investigation under mesophilic temperatures (35 ± 2 °C) for 30 days. The feedstocks were mixed at different percentages according to their availabilities in southern Greece with a total solids (TS) ratio of approximately 10% and an OLR of 2.2 kg VS m^{-3} day^{-1}. The main agro-industrial feedstock of this study is OMW. Two types of influent feedstock were utilized: a mixture of 30% v/v OMW and 70% PM and LPM, and a mixture of 40% v/v of OMW and 60% PM and CW. Therefore, optimization of biogas production from OMW was attempted by co-digesting with PM and LPM or CW. The aim of this study was to investigate the effect of different liquid feedstocks in order to have a total solids (TS) ratio of approximately 10%. Reduction in the volatile solids ranged between 50 and 57%. The average removal of dissolved chemical oxygen demand (COD) was 50% and 58% for the two examined scenarios, while the biogas production rate at the steady state reached 0.7 ± 0.4 and 1.2 ± 0.3 L/L$_{reactor}$/day respectively.

Graphic Abstract
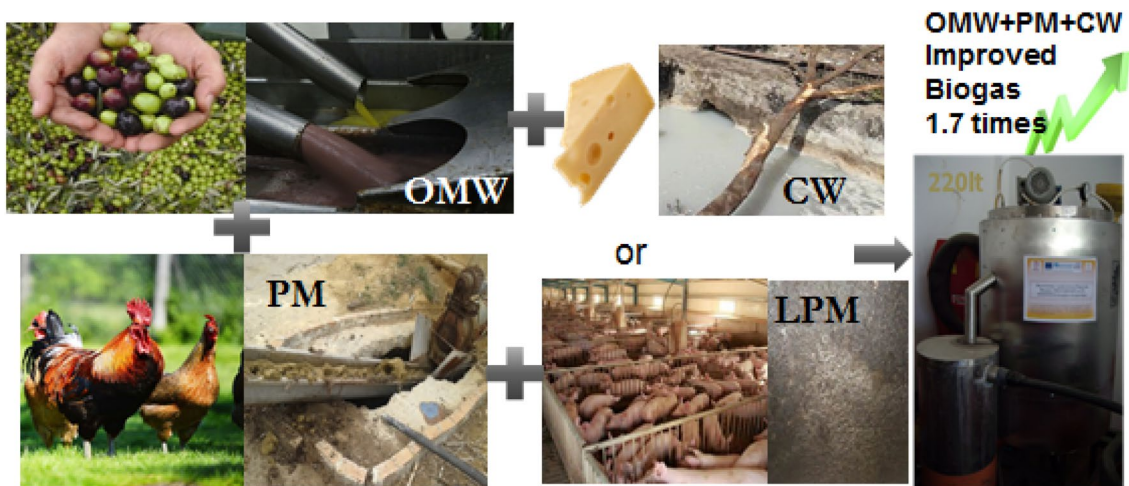
Keywords Agro-industrial by-products · Anaerobic co-digestion · Olive mill wastewater · Cheese whey

Extended author information available on the last page of the article

The Statement of Novelty

The novelty of this work is the introduction of four representative agro-industrial by-products with high organic content and seasonally produced, in Greece to enhance the production of biogas. Raw OMW mixed with varying amounts of PM and different liquid feedstocks (CW or LPM) could facilitate the development of biogas production in other Mediterranean regions with similar sources of organic residues. Greece's key problem is the lack of OMW treatment. Therefore, co-digestion of OMW, PM and CW is an attractive treatment option for these wastes.

Introduction

Agro-industries such as olive oil mills, cheese factories and dairy farms represent a considerable share of the Greek economy. The by-products of three-phase olive oil production such as olive mill wastewater (OMW) and olive cake pose a serious environmental risk. In Greece, the biogas industry has been growing in recent years and more than 10 biogas plants for power production with agro-livestock residues have been established in the last seven years. Furthermore, there are 11 WWTPs treating sewage sludge for biogas production. Germany is the largest producer of biogas in the EU while Greece is near the bottom of the table. Greece's key problem is the lack of an efficient and reliable supply chain. More specifically, the locations of the waste production units for biogas production are scattered, fragmented and, in many cases, almost unknown.

OMW is the liquid fraction of the wastes produced daily during the olive-oil extraction process. The typical range for its chemical oxygen demand (COD) is 50–150 kg/m^3, with extreme values reaching up to 200 kg/m^3 [1]. Due to its high organic content, this wastewater is classified among the strongest industrial liquid wastes and consequently among those with the most significant energy potential. However, the presence of phenolic compounds inhibits methanogenic bacteria [1]. In order to mitigate the effect of phenols on the anaerobic digestion process, co-digestion with other organic residues has been proposed [2].

Since OMW contains a high fraction of organic matter but has a low nitrogen content, the ideal substrate to be used in a co-digestion process would be one that possesses the opposite characteristics. Poultry manure (PM) is an organic material which is rich in nutrients, such as nitrogen, phosphorus and potassium, and traditionally used as organic fertilizer in agriculture. However, its direct application to farmland leads to various environmental problems such as eutrophication in surface waters, pollution of ground water, spread of pathogens, odour and greenhouse gas emissions [3]. In the literature, anaerobic co-digestion of PM and agricultural waste has been investigated using several reactor types and feedstock [4, 5]. The studies have shown that co-digestion of PM and agricultural waste increases the specific methane yield due to better nutrient balance of the substrate mix [4, 5]. For this reason, PM, an ammonium-nitrogen-rich waste, could be a good complementary effluent for the co-digestion of the OMW.

Anaerobic co-digestion of different organic residues has been widely investigated to enhance digestion performance of biogas production and solids reduction [6–8]. The most common co-digestion scenario is that a main basic feedstock (e.g. animal manure or sewage sludge) is mixed with a minor amount of a secondary feedstock (e.g. food waste, glycerin, cheese whey) to feed the digester [9, 10]. Most OMW co-digestion studies have been performed in 100–250 mL serum vials or 0.75–18 L tank reactors [11, 12]. Moreover, several synthetic nutrient, chemical additions and pretreatments (chemical and biochemical) have been reported to enable OMW anaerobic digestion [13–16]. But again, these pre-treatments involve inputs which raise the cost–benefit ratio and also lead to organic load reductions and, consequently, to a decrease of the available methanogenic potential for energy production.

This article focuses on four representative agro-industrial by-products with high organic content and seasonally produced, in Greece and other Mediterranean countries: olive mill wastewater (production period: October–March), cheese whey (production period: January–June), liquid pig manure and poultry manure (production period: whole year). The aim of this work was to examine, on a pilot scale and in continuous experiments, the effect of different waste mixtures available in Crete on methane production for bioenergy generation and to find environmentally friendly and economically feasible solutions to re-use and valorize the majority of agricultural wastes and by-products in Crete, Greece. The specific aim of the present work was to investigate biogas production for raw OMW mixed with varying amounts of PM and different liquid feedstocks (CW or LPM) in order to have a total solids (TS) ratio of approximately 10%. Moreover, the approach and results could facilitate the development of biogas production in other Mediterranean regions with similar sources of organic residues. Different substrate mixing ratios (a mixture of 30% v/v OMW and 70% PM and LPM with a total solids (TS) ratio of approximately 10%, and a mixture of 40% v/v of OMW and 60% PM and CW) were evaluated in order to determine the most effective in terms of achieving the highest methane yield.

Materials and Methods

Agro-Industrial By-Products and Feedstock

Initially, the reactor was inoculated with anaerobic sludge originating from the anaerobic digester of the Municipal Sewage Treatment Plant (MSTP) of the city of Heraklion. Liquid Pig Manure was collected from a local pig farm breeding 70 sows. This facility was fully automated and the generated wastewater was collected in a tank in order to separate the liquid from the solid pig manure. The LPM sample used in our experiments was collected fresh from the tank. Fresh OMW used in the present study was obtained from an olive oil production plant located in Heraklion, which uses a three-phase decanter centrifugation process for the extraction of olive oil. Because of its seasonal production and tendency to ferment, in order to secure a constant feed composition throughout the experimentation period the OMW sample was stored in the freezer at – 18 °C. The cheese whey (CW) was obtained from a local cheese factory located in the same region, using traditional technologies for cheese manufacture. Finally, fresh poultry manure was delivered from a battery chicken farm in Episkopi, Crete.

Wastes were characterized and immediately frozen to avoid biological activity. All feedstock was stored at – 18 °C during the whole experimentation period in order to maintain its physicochemical characteristics. The mean composition of raw LPM, OMW, CW and PM is summarized in Table 1. Although it was expected that freezing might affect the structure of the organic matter favouring the hydrolytic processes due to ice crystal formation, freezing was used as a waste preservation process in order to maintain characteristics as constant as possible. In fact, freezing at temperatures of – 18 °C and below is often preferred to other preservation methods for long-term storage even for food material when freshness is the main criterion for high-quality food standards [17].

OMW and PM were mixed with LPM or CW with a total solids (TS) ratio of approximately 10%. The feedstock characteristics are summarized in Table 2.

Table 2 Characteristics of the influent and effluent from the pilot plant and specific and volumetric methane production from co-substrate A and co-substrate B

Parameters	Co-substrate A		Co-substrate B	
	HRT: 30 days OLR: 2.2 kg VS/m³ day		HRT: 30 days OLR: 2.2 kg VS/m³·day	
	Influent	Effluent	Influent	Effluent
pH	6.9 ± 0.1	7.5 ± 0.4	6.1 ± 0.4	7.4 ± 0.4
TS (g/L)	84.6 ± 9.1	40.1 ± 11.7	85.9 ± 10.9	37.4 ± 7.3
VS (g/L)	59.8 ± 5.9	30.0 ± 6.2	62.1 ± 8.4	26.8 ± 4.1
t-COD (g/L)	90.1 ± 7.5	50.3 ± 12.1	90.3 ± 13.0	52.6 ± 11.3
d-COD (g/L)	42.3 ± 2.7	21.1 ± 6.3	50.9 ± 15.9	19.4 ± 4.7
N (g/L)	6.5 ± 0.5	–	4.4 ± 0.6	–
P (mg/L)	471 ± 92	–	437 ± 86	–
$L_{biogas}/L_{reactor}$/day	0.7 ± 0.4		1.2 ± 0.3	
%CH_4	60 ± 4.7		61 ± 3.4	

Pilot Scale Basic Anaerobic Digester

Laboratory experiments were performed at the Solid Waste & Wastewater Management Laboratory of the School of Agricultural Technology, Technological Educational Institute of Crete. A basic anaerobic digester was manufactured in order to investigate increased biogas production by co-digestion of sewage sludge with agro-industrial by-products. The anaerobic digester was constructed from stainless steel with a double wall. Agitation was ensured by a motor drive unit installed on the top of the reactor. The reactor has a capacity of 220 L with an internal diameter of 60 cm and a height of 80 cm. The reactor was operated under mesophilic conditions (35 ± 2 °C) via an electric water heater and a small pump circulating warm water through a coil of 21 mm poly pipe inside the digester. A top-mounted three-bladed digester mixer was operated at a nominal shaft speed of 100 rpm in digester. Furthermore, the motor was attached to an on/off timer to maintain intermittent mixing of 15 min, twice an hour. The quantity of the biogas produced was recorded daily using a drum-type gas meter, Ritter TG5 model 5.

Table 1 Composition of liquid pig manure (LPM), olive mill waste water (OMW), cheese whey (CW) and poultry manure (PM)

| Parameters	Inoculum	LPM	OMW_A	OMW_B	CW	PM_A	PM_B
pH	7.6 ± 0.2	7.7 ± 0.1	4.9 ± 0.0	5.7 ± 1.6	4.5 ± 0.1	8.9. ± 0.0	8.8 ± 0.1
TS (g/L)	12.3 ± 3.2	9.5 ± 9.6	94.9 ± 2.5	38.9 ± 21.4	73.9 ± 1.1	268.2 ± 3.2	283.9 ± 52.5
VS (g/L)	8.2 ± 2.2	5.9 ± 6.9	83.2 ± 2.4	32.6 ± 17.4	59.9 ± 1.9	180.2 ± 1.6	186.9 ± 19.8
t-COD (g/L)	14.3 ± 4.1	12.9 ± 9.2	195.6 ± 15.3	71.4 ± 22.3	80.7 ± 3.2	7.7 ± 3.3	7.7 ± 3.3
N (g/L)	–	0.2 ± 0.1	0.1 ± 0.01	0.1 ± 0.01	0.6 ± 0.4	20.7 ± 0.4	20.7 ± 0.4

Experimental Procedure and Operational Parameters

Two types of influent feedstock were utilized: a mixture of 30% v/v OMW and 70% PM and LPM, with a total solids (TS) ratio of the mixture approximately 10%, and a mixture of 40% v/v of OMW and 60% PM and CW, in order to investigate the biogas production of olive oil by-products/poultry manure co-digestion. The continuous experiments were carried out in 220 L digester with 180 L working volume. Initially, the reactor was inoculated with anaerobic sludge. Feedstock was added once daily, with a total feeding volume of 6 L daily and a hydraulic retention time (HRT) of 30 days, at a constant temperature of 35 ± 2 °C. The flowchart of experimental procedure is presented in Diagram 1. The co-substrate A was 30% v/v OMW and 70% PM and LPM with a total solids (TS) ratio of approximately 10% with an organic loading rate (OLR) 2.2 kg VS m^{-3} day^{-1} (mean value 2.2 kg VS m^{-3} day^{-1}) (Reactor 1) and the co-substrate B was 40% v/v OMW and 60% PM and CW with a total solids (TS) ratio of approximately 10% with an OLR of 2.2 kg VS m^{-3} day^{-1} (Reactor 2). The digesters were not operated in parallel, so the OMW and PM originated at different periods. Then different characteristics of OMW as feedstock were studied. The digesters were operated for at least 2 subsequent HRTs under steady state conditions characterized by stable biogas production and relatively constant pH throughout the run. Influent and effluent samples were analyzed for TS, VS, pH, T-COD, d-COD and methane content in biogas.

Analytical Methods

The influent and effluent were analyzed for pH and total (TS) and volatile (VS) solids according to APHA (1995) using a pH-meter (Crison, model GLP 21) and appropriate laboratory ovens. Total and dissolved chemical oxygen demand (T-COD and d-COD respectively) and total phosphorus (TP)
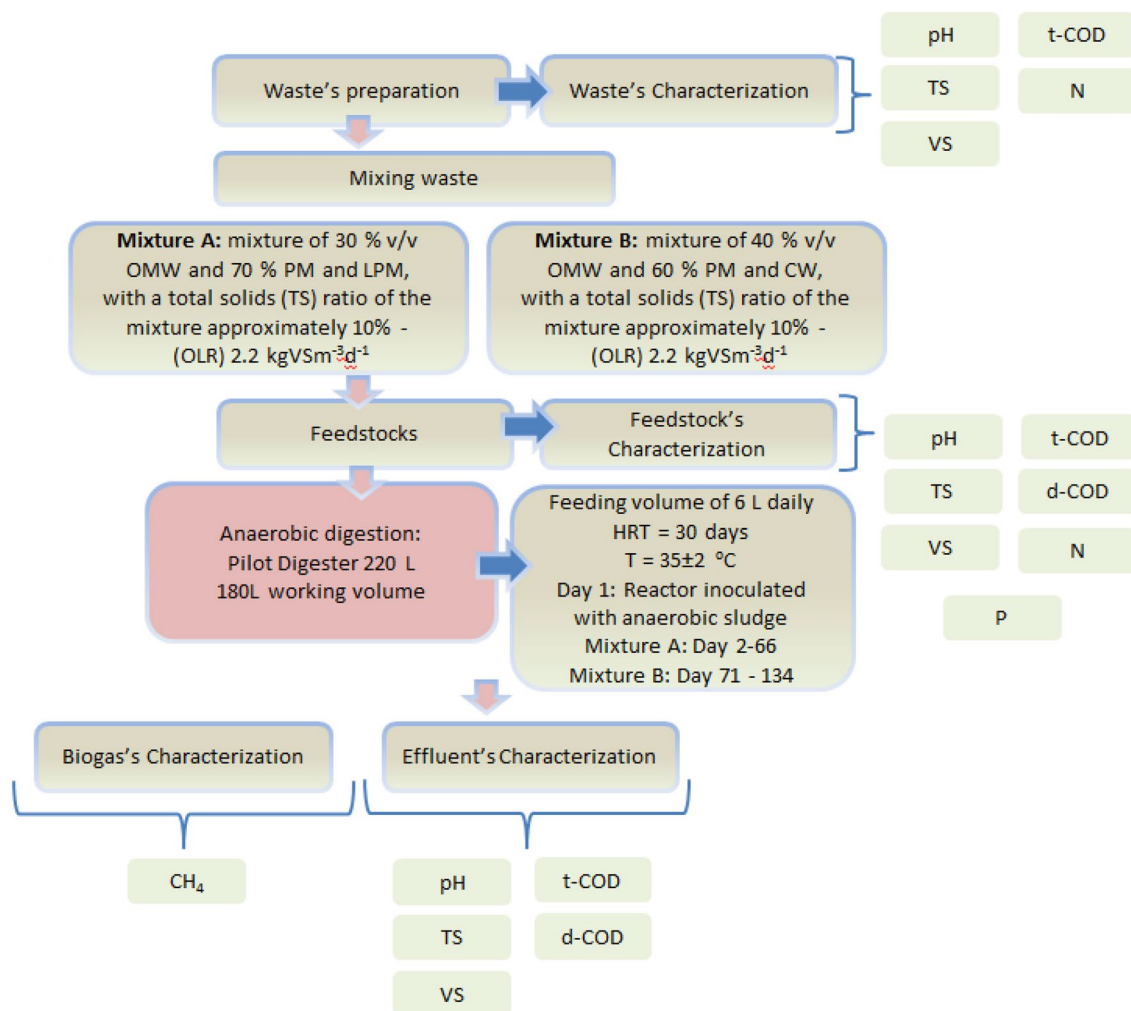
Diagram 1 Flowchart of experimental procedure

were determined spectrophotometrically by use of standard test kits (Hach-Lange). Total kjeldahl nitrogen (TKN) was measured with Semi-Micro-Kjeldahl Method according to standard methods [18]. Biogas yield was monitored continuously by a gas flow meter (Ritter Company, drum type gas meters TG 05).

Biogas composition was analyzed using a gas chromatograph (Agilent 6890 N GC System). Gas samples were collected in gas-tight syringes and transferred to the gas chromatograph by sealing the needle with a butyl rubber stopper. Twenty microliters were injected into a gas chromatograph for methane and carbon dioxide analyses. A thermal conductivity detector (TCD) and a capillary column (GS Carbonplot, 30 m × 0.32 mm, 3 lm) were used. The column was operated isothermally at 80 °C and the detector port was operated at 150 °C. Helium was used as the carrier gas at a flow rate of 15 mL/min. Analyses of all individual samples were carried out in triplicate. The statistical analysis of the data and the results of this study (analysis of average values, variance and standards deviation) were performed using Origin 9 (OriginLab, USA).

Results and Discussion

By-Products Characterization

The primary characteristics of each analysed feedstock are reported in Table 1. OMW and PM were measured twice for co-substrate A and B and selected at different times. The TS, VS, and TCOD were much higher in OMW_A than in OMW_B while PM_A and PM_B present similar characteristics. Significant differences in the composition of the four agro-industrial by-products were detected. OMW_A presents a higher organic content (195.6 g/L as total COD) than the other three (12.9 g/L, 80.7 g/L, 7.7 g/L LPM, CW and PM respectively). OMWA presents a higher organic content than OMW_B (71.4 g/L as total COD). Previous studies reported COD values of OMW between 45 and 170 g/L [19, 20]. The high COD content makes OMW an attractive substrate for biogas production. Nevertheless, as reported by other studies [21–23], the high amounts of phenolic compounds caused inhibition of methanogens. Consequently, this wastewater is not optimal to use alone as substrate for biogas production [24]. Regarding CW in literature [13] reported COD level approximately 75 g/L. The pH values of OMW and CW are significantly acidic, whereas LPM and PM had a pH of 7.7 and 8.9 respectively. Comparable compositional analysis has been reported [9, 19, 25]. OMW, LPM and CW have low nitrogen content, in contrast with PM, so the COD/N ratio is very low for the wastewaters. Comparable compositional analysis has been reported by Borja et al. [15], Orfanoudaki et al. [25] and Dareioti et al. [2]. In particular, their analysis

of total solids (TS), volatile solids (VS) and total chemical oxygen demand (TCOD) was in agreement with our measurements. Mixing of OMW with PM and LPM ensured higher levels of nitrogen in the mixture, whereas organic load of OMW_A was reduced because of OMW 'dilution' with LPM (as shown in Table 2).

Continuous Experiments

Two experiments using CSTR reactors were performed in order to investigate the impact of each mixture on the co-digestion process and determine the most efficient mixing ratio under continuous operation of the reactors. The daily biogas observed in different co-substrates is illustrated in Fig. 1. Figure 1 presents biogas production (L/L$_{reactor}$/day) for mixtures of 30% v/v OMW and 70% PM and LPM and a mixture of 40% v/v of OMW and 60% (PM and CW), with a total solids (TS) ratio of approximately 10% for each mixture. In particular, after co-digestion of 40% v/v OMW and 60% PM and CW, for a hydraulic retention time 30 days, daily biogas increased from 0.7 ± 0.4 L/L$_{reactor}$/day to 1.2 ± 0.3 L/L$_{reactor}$/day, meaning that the increase of OMW and CW co-digestion improved biogas production by 1.7 times. The composition of the methane in the biogas was 60 ± 4.7% for Mixture A and 61 ± 3.4% for Mixture B.

The variation of VS in the inlet and the outlet of the digester for both experimental periods (Mixture A and Mixture B) are presented in Fig. 2. The amount of TS added per day during the operation of the digesters was 8–10%. Therefore, the amount of VS added per day during the operation of the digester with 30% v/v OMW and 70% PM and LPM was 59.8 ± 5.9 g/L, while the amount of VS added after the addition of 40% v/v OMW and 60% PM and CW was 62.1 ± 8.4 g/L. The average concentration of VS in the outlet was found to be 30.0 ± 6.2 g/L during the operation
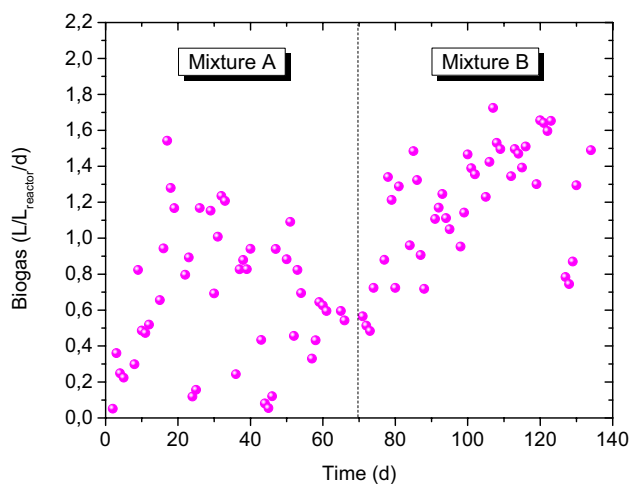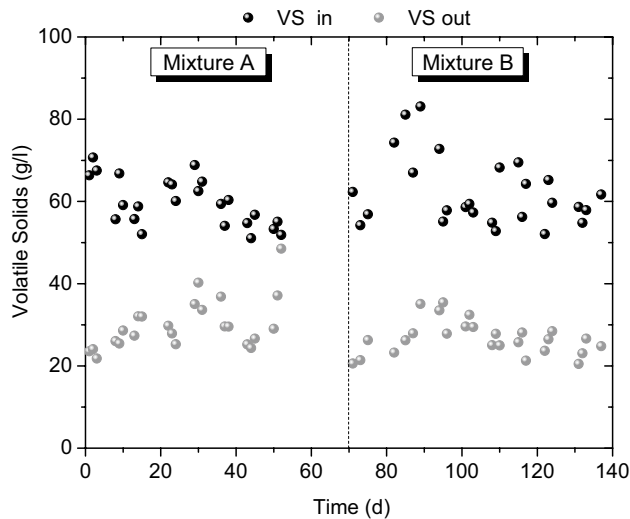

**Fig. 1** Biogas production during the experiments

Fig. 2 VS content variation during the experiments

of the reactor with the mixture of liquid pig manure and 26.8 ± 4.1 g/L during the operation of the reactor with the mixture of cheese whey.

The removal efficiency in VS concentration for the examined scenario of Mixture A and Mixture B was 50% from 59.8 to 30.0 g/L and 57% from 62.1 to 26.8 g/L (Fig. 3) respectively. During the experimentation period of Mixtures A and B, the VS mean concentration of the feed was 59.8 ± 5.8 g/L and 62.1 ± 8.2 g/L respectively. Therefore the VS concentration was almost the same. Mixture B presented a higher VS removal. Maragkaki et al. [9] found a 38.5% VS average removal efficiency when a 95:5 (v/v) mixture of sewage sludge and olive mill wastewater was supplemented. According to Dareioti et al. [2], who co-digested 20% OMW and 80% cow manure, VS reduction was estimated at 34.2%. Furthermore, the co-digestion of SS with OMW (at a ratio
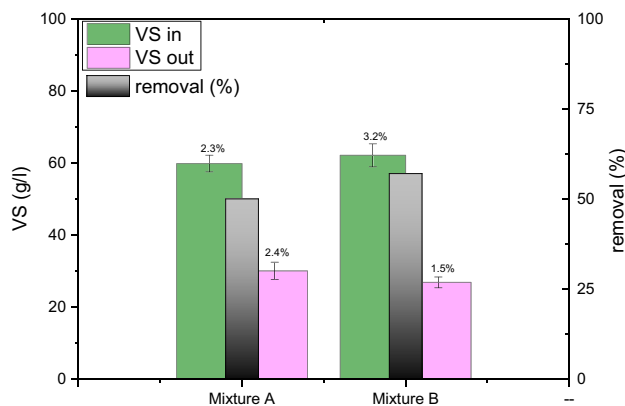
Fig. 3 VS content variation during the experiments. Error bars indicate margin of error with 95% confidence interval
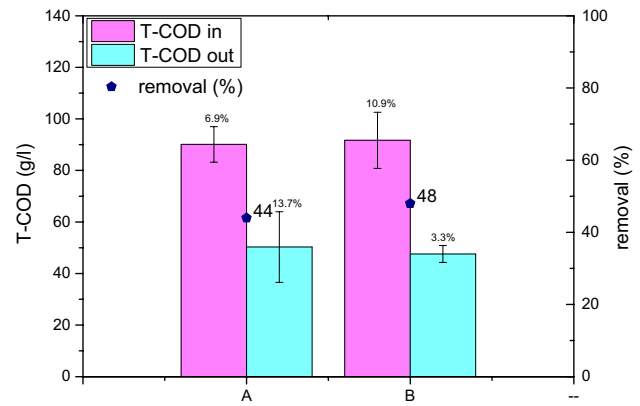

Fig. 4 Mean values of T-COD during the experiments. Error bars indicate margin of error with 95% confidence interval

of 70:30 (v/v)) has a reported VS reduction of 27.6% for HRTs 19.7 d [26].

According to the substrate mass balance model, the VS conversion process could be given by equation (Wang et al., 2019): $QS_{in} = QS_{out} + q_{biogas} Y_{S/B}$, where Q is the flow rate (L/d); S_{in} is the VS concentration in the influent (g VS/L); S_{out} is the VS concentration in the effluent (g VS/L); $Y_{S/B}$ is a coefficient representing the removed substrate transferred into biogas (g $VS_{removal}$/L). According to above equation and Wang et al., 2019 the $Y_{S/B}$ for Mixture A was 1.1367 g $VS_{removal}$/L and for Mixture B was 0.9175 g $VS_{removal}$/L. Based on these coefficients, the biogas yield coefficients for Mixture A and Mixture B were calculated as 0.8797 L/g $VS_{removal}$ and 1.0899 L/g $VS_{removal}$, respectively. These results were very similar to the results reported by Zhang et al. [27] who found that the biogas yield coefficient was 0.912 L/g $VS_{removal}$ when the hydraulic retention time was varied from 20 to 52 days and using chicken manure as the sole substrate.

At standard temperature and pressure (STP), to produce one mol of methane (22.4 L), 2 mol of oxygen-equivalent COD will be destroyed (64 g). In other words, 1 L methane production is equivalent to 2.86 g of COD destruction at STP. In this study, 1 g VS for Mixture A and Mixture B is equivalent to 1.2 g COD and 2.1 g COD, respectively. On this basis, methane production can be related to the reduction in COD and the remainder of the COD removed can be considered to be synthesised biomass [28–30]. When volumetric methane production rate (VMPR) was plotted against volumetric COD removal rate, the VMPR increased with COD removal at a rate of 0.70 and 0.98 g CH4-TCOD g^{-1} $TCOD_{removed}$ at Mixture A and Mixture B, respectively. Thus, about 30% and 2% of the COD removed was converted to biomass at Mixture A and Mixture B, respectively.

The total COD removal during the examined scenarios was 44% from 90.1 ± 6.1 to 50.3 ± 12.1 g/L for Mixture A and 48% from 91.7 ± 13.6 to 47.6 ± 4.1 g/L, mean value,
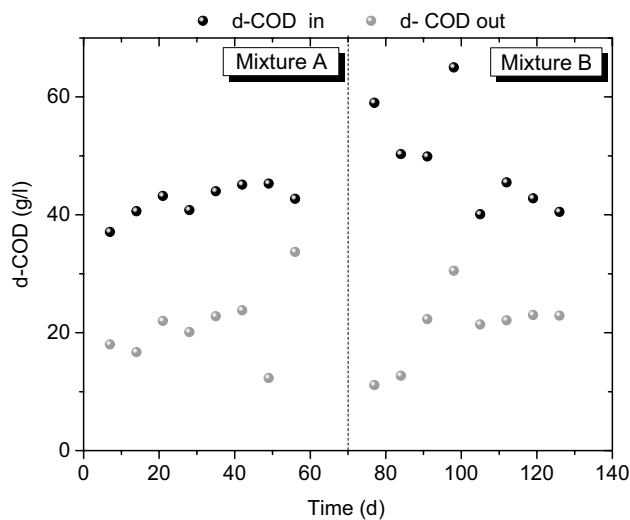
Fig. 5 Profile of d-COD during anaerobic digestion process of mixture A and B

Fig. 6 pH variation during the experimental periods

during OMW, CW and PM (Mixture B) co-digestion (Fig. 4). The d-COD removal during the examined scenarios was 50% (mean value) from 42.4 to 21.2 g/L and 58% (mean value) from 49.1 to 20.8 g/L (Fig. 5) during OMW&PM&LPM and OMW&PM&CW respectively. The best T-COD and d-COD removal efficiencies of approximately 48% and 58% respectively were achieved for Mixture B, CW addition. The removal of COD in conjunction with gas production in the reactor provided evidence of effective microbial activity by methanogenic bacteria. A total COD removal of 50% by digesting a feed consisting of a mixture of 80% and 20% OMW under mesophilic conditions and a hydraulic retention time of 19 days has been shown [2] which is in accordance with our results for OMW (48%).

The rapid increase in biogas production was supported by a simultaneous decrease of COD (Fig. 4 and 5). The high organic load in OMW mandates its mixing with other industrial wastewaters in order to digest them successfully and eliminate the environmental risks of no treatment at all.

The pH values in the feedstocks and the digester are presented in Fig. 6. During the experimentation period of Mixture A, the pH in the reactor was stable between 6.7 and 8.3 (7.5 ± 0.4 mean value), while the influent value was about 6.7 and 7.1 (6.9 ± 0.1 mean value). The pH value of CW was 4.5 ± 0.1. In consequence, the addition of CW to the mixture of OMW & PM decreased the pH in the feedstock compared to LPM addition, which pH value was 7.7 ± 0.1. During the OMW&PM&LPM co-digestion, the pH in the reactor was approximately stable between 6.5 and 7.9 (7.4 ± 0.4 mean value), while the influent value was about 4.8 and 6.7 (6.1 ± 0.4 mean value). pH value is known to play an important role during anaerobic digestion as it affects the solubilization process [31], creating a favourable environment
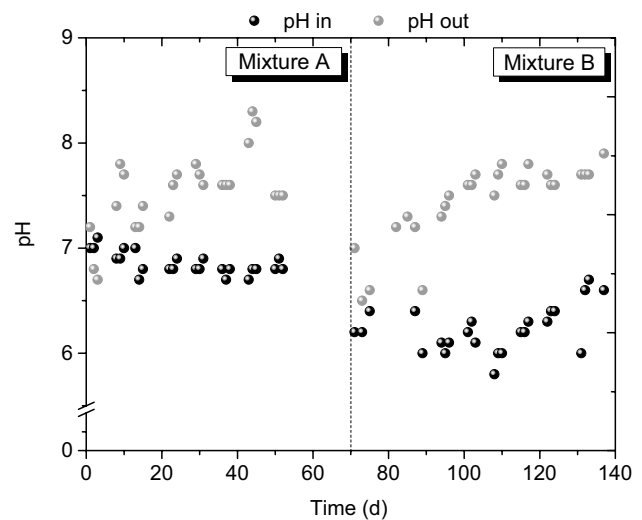
for microbes [32] and influencing the enzymatic reactions [33]. Previous researchers reported that the optimum pH for maximum methane production should be between 6.8 and 7.2 [34]. The addition of LPM improved the pH value of the feedstock while the CW addition decreased the pH in the feedstock. The pH value in the digester was stable with the addition of LPM and CW and the pH presented values near the optimum for maximum methane production. However, the optimum values appeared with the addition of CW in the mixture of OMW & PM.

Conclusions

The specific aim of the present work was to investigate biogas production for raw OMW mixed with varying amounts of PM and different liquid feedstocks (CW or LPM) in order to have a total solids (TS) ratio of approximately 10% and an OLR of 2.2 kg VS m^{-3} day^{-1}. Different mixing ratios of the substrates were evaluated, in order to determine the most effective in terms of achieving the highest methane yield. Two types of influent feedstock were utilized: a mixture of 30% v/v OMW and 70% PM and LPM and a mixture of 40% v/v of OMW and 60% PM and CW. The experiments showed that after co-digestion of 40% v/v OMW and 60% PM and CW, daily biogas increased from 0.7 ± 0.4 L/L$_{reactor}$/day to 1.2 ± 0.3 L/L$_{reactor}$/day, meaning that the increase of OMW and CW co-digestion improved biogas production by 1.7 times. Reduction in the volatile solids ranged between 50 and 57% while the average removal of dissolved chemical oxygen demand (COD) was 50% and 58% for the two examined scenarios. Therefore, co-digestion of OMW, PM and CW is an attractive treatment option for these wastes,

because manure improves the buffer capacity of the mixture and a high methane yield can be achieved.

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