Biogas: olive mill wastewater as complementary substrate of piggery effluent

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ABSTRACT: The anaerobic digestion of a peculiar piggery effluent (PE), with a high organic content (93 g/L), was carry out using olive mill wastewater (OMW) as complementary substrate. From the different tested conditions – [100%PE], [70%PE+30%OMW], [50%PE+50%OMW], [20%PE+80%OMW] – units containing only PE and the lowest proportion of OMW in the mixture (30% OMW), provided the highest biogas volume of about 780 mL (70% CH_4). Comparatively, identical quantities of each substrate ([50%PE+50%OMW]) generates some gas (320 mL, 60% CH_4), understood as the result of an adaptation process by the microbial consortium, while the [20%PE+80%OMW] condition provided even less gas volume (120 mL, 6% CH_4), probably due to the antimicrobial capacity of the phenolic compounds in OMW, confirming the negative influence of using so high OMW proportion.

1 INTRODUCTION

Portugal owns large breeders (at least 400 pigs and 100 sows) which manage more than two thirds of the pigs fattened before slaughtering, being in a similar situation to countries such as the Czech Republic, Estonia, Ireland, Greece and Cyprus (EUROSTAT 2014). The production of pigs in Portugal was about 2,165,000 heads in 2017, counting for 1.2% of the Europe production (FAOSTAT 2019). This activity produces high volumes of piggery effluent, which is an unbalanced and, consequently, a potential inhibiting substrate to the anaerobic digestion process, mainly due to high ammonia concentrations. A huge research was done in the scope of inhibiting recalcitrant effluents, involving several arrangements of various pre-treatments processes, which result in very expensive procedures not always achieving good results. The concept of "effluents complementarity" was study and successfully applied to organic effluents by anaerobic digestion, as the case of the OMW treatment. The OMW was digested without chemical correction or pre-treatment by means of a feeding strategy involving an OMW fraction of 83% (v/v) in admixture with complementary substrate (Marques 2001; Gonçalves et al. 2012). Sampaio et al. (2012) confirmed that the drawbacks of the raw OMW characteristics could be easily overcome by applying the same feeding approach through heterotrophic microalgae (*Chlorella protothecoides*) as OMW complementary effluent. Later on, Assemany et al. (2018) reported the beneficial effect of substrates complementarity on anaerobic processes since the replacement of algal biomass by OMW (10%, v/v) provided a threefold increase in methane production compared to algal digestion alone. According to Sampaio et al. (2011), the use of complementarity concept allows to obtain a stable anaerobic digestion able to degrade OMW in its original composition (100%, v/v), making the process simpler, more flexible and cheaper. The objective of this work was to optimize operational conditions of the anaerobic digestion of a peculiar high organic content piggery effluent by means of the effluent complementarity concept, using OMW as complementary substrate.

2 MATERIALS AND METHODS

2.1 *Substrates*

The substrates assayed were: piggery effluent (PE) collected in *Valorgado* Company (Salvaterra de Magos, Portugal), using the liquid fraction after main solids removal by a solid-liquid separator, and olive mill wastewater (OMW) collected from an olive mill (Rio Maior, Portugal) using three-phase continuous extraction process.

2.2 *Anaerobic digestion experimental set-up*

Anaerobic digestion assays were carried out in batch mode, under 37±1ºC, by using glass reactors with 165 mL total volume. Tested conditions were run in triplicate as designated in Table 1.

Table 1. Scope of the anaerobic digestion experiment.

Substrates composition (%, v/v)		
PE	OMW	Designation
100	0	[100%PE]
70	30	[70%PE+30%OMW]
50	50	[50%PE+50%OMW]
20	80	[20%PE+80%OMW]

2.3 *Analytical and chromatograph methods*

Performance of the process was monitored by analytical characterizations of all samples and by the volume and quality of the obtained biogas. Total and volatile solids (TS, VS), chemical oxygen demand (COD), total nitrogen (Kjeldahl, TN), ammonium (NH_4^+-N), and pH, were assayed according to Standard Methods (APHA 2012). Biogas production was monitored by means of a pressure transducer, while the gas composition and volatile fatty acids (VFA) were analysed by chromatographic techniques (Varian 430-GC, TDC; HP-5890, FID, ASTM Standard Method 2000). The characterization of substrates is shown in Tables 2-4.

Table 2. Effluents characteristics: COD and solids

	COD (g/L)	Total solids (g/L)	Volatile solids (g/L)	Total nitrogen (mg/L)	Ammonium nitrogen (mg/L)
PE	93 ± 5	47.4 ± 0.79	31.9 ± 0.6	4900 ± 277	3206 ± 20
OMW	106 ± 1	31.8 ± 0.04	26.1 ± 0.2	213 ± 16	1.4 ± 1.6

Table 3. Effluents characteristics: pH values and volatile fatty acids

	pH	Acetic acid (mg/L)	Propionic acid (mg/L acetic)	Isobutyric acid (mg/L acetic)	Butyric acid (mg/L acetic)	TOTAL VFA (mg/L acetic)
PE	7.27	1373.0	561.8	2157.7	1545.7	5638.2
OMW	5.09	213.0	64.0	110.4	159.5	546.9

Table 4. Effluents characteristics: phenols and antioxidant activity

	Total phenols	Antioxidant activity	
	(g GAE/L)	(mmol TEAC)	DPPH Inhibition (%)
PE	0.89 ± 0.001	1.11 ± 0.012	80.9 ± 0.70
OMW	3.12 ± 0.017	0.80 ± 0.069	60.9 ± 2.97

2.4 *Quantification of total phenolics content*

Total phenolics content was determined by the Folin-Ciocalteu colorimetric method (Singleton & Rossi 1965), according to an improved procedure described by Hagerman et al. (2000). Total phenols were expressed as g GAE (gallic acid equivalents)/L by comparison to the gallic acid standard curve. Results were obtained in triplicate.

2.5 *DPPH radical scavenging assay for Antioxidant activity evaluation*

Radical scavenging activity against stable DPPH radical (2,2-diphenyl-2-picrylhydrazyl hydrate) was determined spectrophotometrically. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep-violet to light-yellow) were detected at 515 nm on a UV/visible light spectrophotometer. Radical scavenging activity of samples was measured by a modified method (Brand-Williams et al. 1995). The decreasing of the DPPH solution absorbance indicated an increase of the DPPH radical-scavenging activity. The experiment was carried out in triplicate. Radical scavenging activity (%) was calculated by the equation 1.

$$\%\text{DPPH inhibition} = [(Abs_b - Abs_f)/\ Abs_b]\ \times 100 \qquad (1)$$

where Abs_b is the absorption of blank (t=0 min) and Abs_f is the absorption of tested solution (t=30 min).

The antioxidant activity was expressed in mmol TEAC (Trolox Equivalent Antioxidant Capacity), by preparing a standard curve using Trolox as an antioxidant standard.

3 RESULTS AND DISCUSSION

3.1 *Effluents*

This particular piggery effluent used is an unusual very concentrated substrate, whose organic matter content resembles OMW concentrations. So, the presence of such high organic concentrations (93-106 g/L COD: Table 2) indicates that there is a great potential for biogas/methane production. On the other hand, this effluent presents complementary characteristics in terms of composition that can be used advantageously to balance the suitable conditions of the anaerobic digestion process. Effectively, the inhibitory capacity of OMW, due to the total phenols concentration (≈ 3 g/L), associated with the acid pH (5.0) (Tables 3 and 4), can be minimized by piggery effluent addition. Furthermore, OMW nitrogen absence (evaluated through total and ammonia nitrogen contents) may also be compensated by the presence of the high nitrogen content of piggery effluent (4.9 g/L total, 3.2 g/L ammonium nitrogen: Table 2).

3.2 *Biogas/methane production*

Biogas production was registered in all tested mixtures without any "lag" phase (Figure 1).

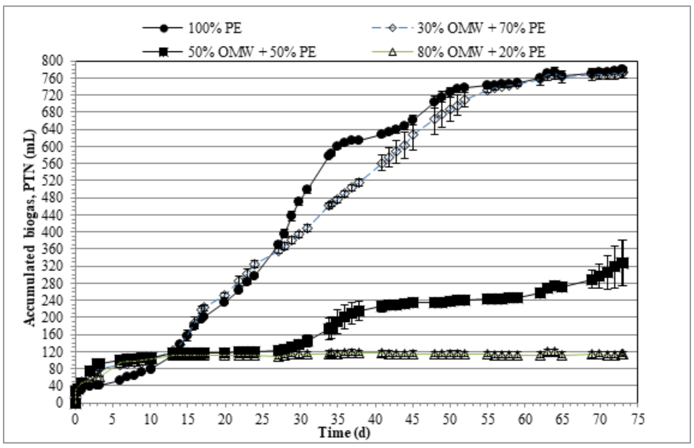

Figure 1. Biogas production

The biogas production started immediately and a similar accumulated volume (≈ 120 mL) was observed in all units, elapsed 13 days. From then on, and over the remaining experimental time, units containing [100%PE] and [70%PE+30%OMW] showed a similar behaviour, providing the highest accumulated biogas amount obtained (≈ 780 mL). For the other two trials, only by the 30th experimental day, the assay [50%PE+50%OMW] was able to generate some gas but it did not exceed the average value of 320 mL. This behaviour can be understood as the result of an earlier process (before the day 30^{th}) of microorganism adaptation to the effluents mixture, which allowed further increases in gas production. Comparatively, the gas production absence from the assay involving a volume participation of 80% OMW, suggests that under the tested operating conditions, around 50% v/v is the limiting OMW quantity in the blend. Concerning biogas composition, the units [100%PE] and [70%PE+30%OMW] showed the highest methane concentrations (around 70% CH_4: Table 5), being in conformity with the previous observations and indicating the presence of a healthy methanogenic archaea population in both assays.

Table 5. Biogas composition

Substrates	Methane (% v/v)	Carbon dioxide (% v/v)
[100%PE]	71.00 ± 0.26	29.00 ± 0.26
[70%PE+30%OMW]	70.27 ± 0.57	29.73 ± 0.57
[50%PE+50%OMW]	60.30 ± 6.68	39.70 ± 6.68
[20%PE+80%OMW]	5.57 ± 8.86	94.43 ± 8.86

The value of 60% methane in biogas from [50%PE+50%OMW] mixture may result from the already referred adaption process of the remaining consortium that had maintained the capacity of converting the substrate and produce biogas/methane. As expected, a very poor biogas was obtained in [20%PE+80%OMW], confirming the negative influence on anaerobic consortium if so high OMW proportions are included in the influent.

3.3 *Removal capacity*

The highest amount of organic material removal was observed, as expected, in the units [100%PE] and [70%PE+30%OMW], with [100%PE] being less efficient than [70%PE+30%OMW]. Removal values of 63% against 75% of initial COD (93 and 81 g/L) were respectively reached (Table 6). Solids concentrations of 40-47 g/L TS and 28-32 g/L VS ([100%PE] and [70%PE+30%OMW] units (Table 6) were removed in proportion of 17-21% (TS) and 30-36% (VS).

Table 6. Chemical oxygen demand and solids

Substrates	COD		Total Solids		Volatile Solids	
	initial (g/L)	removal (%)	initial (g/L)	removal (%)	initial (g/L)	removal (%)
[100%PE]	93 ± 5	62.5	47.4 ± 0.79	16.5	31.9 ± 0.56	29.5
[70%PE+30%OMW]	81 ± 3	74.6	39.6 ± 0.04	21.0	27.8 ± 0.03	35.6
[50%PE+50%OMW]	77 ± 3	47.6	38.0 ± 0.05	16.1	27.7 ± 0.00	22.0
[20%PE+80%OMW]	73 ± 1	28.9	32.8 ± 0.60	20.7	25.4 ± 0.61	25.2

VFA contained in digesting substrate of [100%PE] and [70%PE+30%OMW] were more efficiently converted than the other and removal values of 85 and 56% were registered, respectively (Table 7). As known, most of the methane formed in the anaerobic digestion process results from the acetic acid. In the two present cases, the contribution of acetic acid in total of VFA at baseline was 24 and 58%, meaning that the remaining VFA correspond to higher-chain acids than acetic and of more difficult degradation, such propionic and butyric (data not shown), justifying the slower initial gas production of these units (Figure 1). This situation presumes the existence of a buffer capacity in the medium capable of maintaining the process balance, for which the high amounts of nitrogen in effluents had certainly a positive effect on the digestion process. Initial nitrogen amount (total and ammonia nitrogen; Table 8) decreased with PE decreasing volume, as expected, due to the presence of OMW, characterized by low nitrogen levels.

Table 7. Volatile fatty acids

Substrates	Acetic acid (mg/L) initial	Acetic acid (mg/L) final	Total VFA (mg/L acetic) initial	Total VFA (mg/L acetic) final	(%) removal
[100%PE]	1373	688	5638.2	831.4	85.3
[70%PE+30%OMW]	2314	1190	3962.0	1761.5	55.5
[50%PE+50%OMW]	2175	2061	3683.9	6675.5	-81.2
[20%PE+80%OMW]	1801	757	2792.2	1328.5	52.4

Table 8. Nitrogen

Substrates	pH initial	pH final	Total nitrogen (g/L) initial	Total nitrogen (g/L) final	Ammonia nitrogen (g/L) initial	Ammonia nitrogen (g/L) final
[100%PE]	7.27	8.05	4.90 ± 0.280	n/d	3.21 ± 0.019	3.86 ± 0.158
[70%PE+30%OMW]	6.90	7.94	1.83 ± 0.024	1.84 ± 0.075	1.35 ± 0.113	2.55 ± 0.297
[50%PE+50%OMW]	6.66	7.25	1.35 ± 0.004	1.32 ± 0.024	0.83 ± 0.029	0.95 ± 0.012
[20%PE+80%OMW]	6.23	5.73	0.58 ± 0.012	0.58 ± 0.039	0.34 ± 0.014	0.29 ± 0.004

Initial concentrations of total nitrogen were more or less maintained after anaerobic digestion while those relative to ammonia nitrogen were increased due to protein material degradation during the process. The only exception concerns the digestions with the highest proportions of OMW (80%), which always had the worst results and where the decrease of ammonia nitrogen content confirms the deficient anaerobic digestion functioning, indicating an insufficient activity to perform the degradation/conversion of the organic matter. Accordingly, this mixture emphasized its acidic characteristics during digestion, in which the pH evolved from 6.2 to 5.7 (Table 8), testifying the presence of an imbalanced process in the [20%PE+80%OMW] unit, because of the inhibitory conditions establishment. In the other assays, the anaerobic process led to increased pH values, in a range close to neutrality (6.7-7.3 to 7.3-8.1).

The phenolics content (Total phenols, TP; Table 9) rise as the PE amount decreases in the initial substrates, indicating that OMW is mainly responsible for this increase and, subsequently, the highest TP content was found in admixtures containing OMW. The opposite is observed in relation to the antioxidant activity (Table 9). Doesn't seem to be significant difference for the initial values of the antioxidant activity (ranging 0.9-1.1 mmol TEAC and 68-81 % DPPH radical inhibition). One should bear in mind that different type of phenolics compounds is involved in both PE and OMW, and also, total phenolic content doesn't necessarily correlates with antioxidant activity. Nevertheless, after digestion, it was verified that the lowest antioxidant values were observed in units with highest PE amount ([100%PE] and [70%PE+30%OMW]).

Table 9. Total phenols and antioxidant activity

Substrates	Total phenols (g GAE/L) initial	Total phenols (g GAE/L) final	Antioxidant activity (mmol TEAC) initial	Antioxidant activity (mmol TEAC) final	DPPH Inhibition (%) initial	DPPH Inhibition (%) final
[100%PE]	0.89±0.001	0.86±0.001	1.11 ± 0.01	0.64 ± 0.10	80.9 ± 0.70	30.3 ± 8.32
[70%PE+30%OMW]	1.69±0.001	1.10±0.000	1.07 ± 0.02	0.39 ± 0.08	78.6 ± 0.29	9.7 ± 6.58
[50%PE+50%OMW]	2.18±0.000	1.52±0.003	0.99 ± 0.02	1.34 ± 0.03	73.7 ± 1.37	69.1 ± 1.66
[20%PE+80%OMW]	2.69±0.002	2.50±0.001	0.91 ± 0.01	1.35 ± 0.02	68.1 ± 0.21	69.5 ± 1.50

TP concentrations of 1.7 and 2.2 g/L were removed in proportions of 30 and 35% for [70%PE+30%OMW] and [50%PE+50%OMW], respectively, while [20%PE+80%OMW] unit presented a poorer removal amount (7%) after digestion. No significant change was observed for TP concentration in piggery digestion. However, the antioxidant activity decreased to nearly half of the initial value, revealing that most likely, other compounds than phenolics in PE, which are degraded during digestion, may be responsible for this activity. Therefore, these decrease in antioxidant activity for the anaerobic effluent of [100%PE] and [70%PE+30%OMW], indicates the loss of compounds of interest, however, the beneficial characteristics associated with these parameters were still evident in the digested material. In the two other assays ([50%PE+50%OMW] and [20%PE+80%OMW]), there was a maintenance or even an increase of the initial values for the antioxidant activity, revealing the importance of the OMW composition for this parameter. From the results obtained, one can infer that it is possible to valorise energetically this peculiar PE, alone or in admixture with a small amount of OMW, through the biogas production, and take additional advantage of the remaining compounds of interest present in the digested material.

4 CONCLUSIONS

Piggery effluent can be degraded under anaerobic conditions alone or in admixture with 30% OMW (v/v). The highest gas volumes (≈ 780 mL biogas, 70% CH_4) were obtained in both experiments. OMW is a substrate with high inhibiting capacity against the anaerobic digestion probably due to the known antimicrobial capacity of phenolic compounds in it, and only a small fraction such as 30% (v/v) can be added to PE without damaging the process.

The low biogas production obtained after using a proportion of 50% (v/v) OMW in the blend confirms the inhibitory characteristic of this substrate. However, it was observed that microbiota has the capacity to adapt to substrate disadvantageous characteristics. Then under the same operational condition, a possible extension of experimental time could provide better results. From the available data, there is no relevant advantage in associating OMW with this peculiar PE.

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