EVALUATION AND MODELLING OF METHANE YIELD EFFICIENCY FROM CO-DIGESTION OF WASTE ACTIVATED SLUDGE AND OLIVE MILL WASTEWATER

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Abstract. This research aims to enhance the biodegradation efficiency of waste activated sludge co-digested with olive mill wastewater in a batch system at a laboratory scale in (600 ml) digester. The potential biomethane production was investigated. Different concentration ratios were tested out at a thermophilic temperature (55°C) for a retention period of 32 days. The results showed an increase in methane amount in the case of co-digestion. A height methane yield was obtained (71% of CH_4) at a mixing ratio: 87.5/12.5 of waste activated sludge/olive mill wastewater. The kinetic modelling was done to analyze the digestion performance with two models: the modified Gompertz equation and the modified logistic equation. The kinetic data and the concentration ratio give a peak correlation, whose the Gauss amplitude equation is convenient to predict the optimum mixing ratio and the limited concentration to avoid the inhibition of process. The synergistic effect is limited if olive mill wastewater mixing ratio exceeds the limited ration (22%).

Keywords: *anaerobic co-digestion, waste activated sludge, olive mill wastewater, kinetic study, synergistic effect*

Introduction

Anaerobic digestion is a waste management method aimed at the reduction of harmful effects on the environment (Manyi-Loh et al., 2013; Ali Shah et al., 2014). Anaerobic digestion (AD) has been recognized as an efficient bioprocess for the management of waste activated sludge (WAS) (Kardos et al., 2011), by offering many environmental and economic benefits (Mulat and Horn, 2018). In this method, microorganisms play a crucial role in treating organic matter and returning the chemical elements into the active cycle. Thus, they are effective in mineralization of the complex organic matter through a sequential breakdown and release of chemically stabilized compounds, mainly methane (CH_4) and carbon dioxide (CO_2) (Gopinath et al., 2014; Ali Shah et al., 2014; Zeng et al., 2017). However, WAS is known for its low biodegradability mainly due to its low carbon to nitrogen (C/N) ratio. This limits its digestion under traditional mesophilic conditions (Coelho et al., 2011; Mahanty et al., 2014). Therefore, the co-digestion of sludge with other organic wastes can offer numerous potential benefits for the AD process, such as: dilution of the potentially toxic compounds eventually existing in any treated materials, augmenting the organic matter biodegradability, better biogas yield due to synergistic effects, tuning of the moisture content and pH, strengthening the essential buffer capacity to the mixture and the enlarging of bacterial range strains taking part in the process (Anjum et al., 2017; Kashi et al., 2017; Xu et al., 2018).

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Therefore, notable issues have been performed by digesting simultaneously the WAS with different biological wastes (Heo et al., 2004; Bolzonella et al., 2006; Carrere et al., 2008; De Vrieze et al., 2013; Sun et al., 2013; Qiao et al., 2015; Mulat and Horn, 2018).

Olive Mill Wastewater (OMW) is a very attractive co-substrate option for the anaerobic co-digestion of municipal sludge because, carbon source addition like OMW as a substrate, enhanced the total VS and therefore the biogas yield (Ma et al., 2008). OMW is becoming a serious environmental problem, especially for its high chemical oxygen demand (COD). It is generally acknowledged that the high toxicity of OMW is entirely ascribable to phenols (Perez et al., 1992).

This article focuses on the anaerobic co-treatment of two typical wastes in Algeria, which are totally unexploited and in some cases harmful to the environment. WAS (production period whole year) and OMW (production period October–March) and as two representative types of biomass wastes produced in Boumerdes (Algeria) and other mediterranean countries. The precise aim of the present research was to investigate biochemical methane potential assays for raw WAS alone and mixed with varying amounts of OMW.

Material and methods

Waste sampling

The sampling of WAS was carried out in a municipal wastewater treatment plants in Boumerdes (Geographical coordinates are 36°45'0"N and 3°40'0"E in DMS), Algeria. When the sludge age, is 12 days in the extended aeration. The OMW used in this study was taken from a three-phase olive mill processing plant located at the Issers city (Geographical coordinates are 36°43'0"N and 3°40'0"E in DMS) in Boumerdes during the harvesting season. The biochemical compositions of wastes are revealed in *Table1*.

Table 1. *Characteristics of substrate*

Parameters	Waste activated sludge	Olive Mill Wastewater
pH	7.8 ± 0.15	4.8 ± 0.1
CODt (g l^{-1})	90.7 ± 1.8	128.1 ± 5.4
CODs (g l^{-1})	35.0 ± 0.6	64.7 ± 1.4
TS (g l^{-1})	150 ± 0.8	69.5 ± 3.1
VS (g l^{-1})	71.7 ± 0.5	57.4 ± 4.5
TN (g l^{-1})	3.8 ± 1.8	1.26 ± 2.2
TP (g l^{-1})	0.903 ± 0.07	0.48 ± 0.09
TPc (eq gallic acid. g l^{-1})	/	4.11 ± 0.3
Oil and grease (g l^{-1})		17.4 ± 1.7
Cd (mg l^{-1})	201	$<1\times10^{-3}$
Cr(mg l^{-1})	508.9	0.655
Pb (mg l^{-1})	335.5	0.186
Mn(mg l^{-1})	922.5	$<1\times10^{-3}$
Ni (mg l^{-1})	$<1\times10^{-3}$	3.96×10^{-2}
Fe(mg l^{-1})	4520	1.504
Zn(mg l^{-1})	30.63	0.24
Cu(mg l^{-1})	1116	0.33

CODt total chemical oxygen demand , CODs: Soluble chemical oxygen demand
TS :total solids, VS: Volatile solids , TN:total nitrogen / TP total phosphorus
TPc: total phenolic compounds

Digester and operation

A 600 ml digester used for producing biogas from biomass through AD (*Figure 1*). The functioning volume of each digester was maintained at 450 ml and run under uncontrolled pH. For these experiments, the inoculum was an anaerobic sludge treating WAS which was diluted to 0.64 g/l of volatile solids (15 ml /for each digester). When the mixtures were prepared at different weight ratios (WAS % / OMW %): (87.5 / 12.5, 75 / 25, 50 / 50, 25 / 75, 12.5 / 87.5) and the mono- digestion of both wastes (100/0, 0/100), the bioreactor was purged with helium gas to eliminate air from the reactor. Experiments were carried out at a thermophilic temperature of 55°C by incubation in Marie-bath. Biogas volume generated was measured by liquid displacement (NaOH 2%) (Esposito et al., 2012). The chemical compositions of each mixture ratio are revealed in *Table 2*.

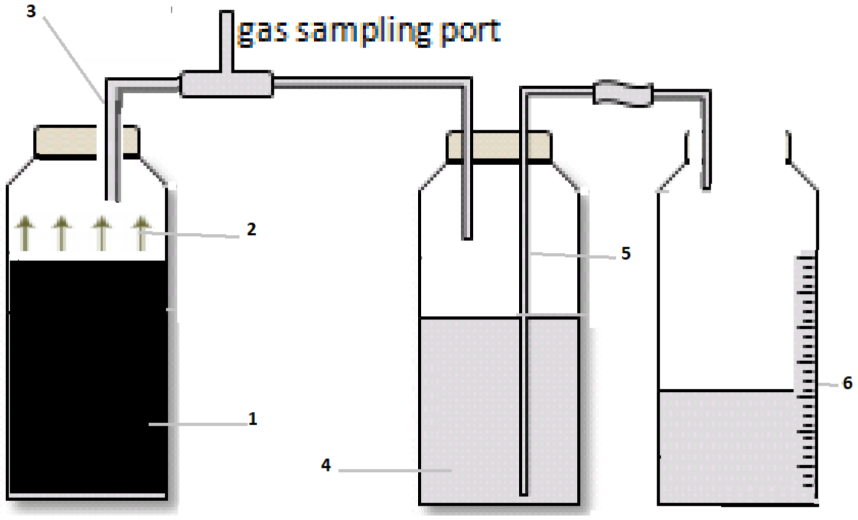

Figure 1. *Anaerobic digestion system: From each digester placed in a water bath (1) a silicone tube (2) led the generated gas (3) out. This tube was led to the top of another glass bottle which contained NaOH 2% solution (4). There was another tube (5) from the bottom of that bottle, through which the gas pumped the solution to the graduated bottle meter (6). This way, it was possible to measure the volume of the generated gas accurately, on a daily basis*

Analytical method

Soluble and total chemical oxygen demand (CODt and CODs) total nitrogen (TN) and total phosphorus (TP), Total solids (TS) and Volatile solids (VS) were quantified according to Standard Methods (Apha, 1998). The pH of the wastes measured according to NF ISO 10390 by a portatif pH-Metre (HANNA HI8424, France) (Rodier et al., 2009). Total phenolic compounds (TPc) were extracted and purified in ethyl acetate using the method of Macheix et al. (1990). The concentration of TP compounds was determined spectrophotometrically (according to the Folin–Ciocalteu method (Singleton and Rossi, 1965)) Heavy metals were determined by the atomic absorption spectrophotometer (Perkin Elmer, Optima8000) according to the Method cited by Liu et al. (2001). The biogas composition (CH_4 + CO_2) was measured using a gas chromatograph (GC-HP 5890) coupled with a thermal conductivity detector (*TCD*) and stainless steel column that was 2 m long with a 5 mm OD and 2 mm ID and contained Porapak Q 100 that had a mesh range from 80 to100. The carrier gas was N_2, and the analysis was carried out at a carrier gas flow rate of 30 ml min^{-1} with the injector, column, and detector temperatures at 120, 90, and 120ºC, respectively.

GC-MS analysis of ethyl acetate extract of OMW was performed on a BRUKER SCION 365 GC System (NS-GC 1409S312), Gas chromatography (GC) coupled to a triple quadruple mass spectrometer fitted with an Rtx-5MS capillary column (30 m X 0.25 mm inner diameter, X 0.25 µm film thickness; maximum temperature, 350°C). Ultra-high purity helium (99.99%) was used as a carrier gas at a constant flow rate of 1.0 ml min^{-1} using the condition cited by Al Owaisi et al. (2014). The percentage composition of the ethyl acetate extract components was expressed as a peak area percentage. The identification and characterization of chemical compounds was based on GC retention time. The mass spectra were computer matched with those of standards available in mass spectrum libraries.

Table 2. *Characteristics of the mixture prepared with different waste activated sludge/Olive Mill Wastewater mixing ratios*

WAS /OMW ratios	TS (g l^{-1})	VS (g l^{-1})	CODt/TN	pH
100 /0	150± 0.8	71.7± 0.5	18.95	7.8± 0.15
87.5 /12,5	138.78± 0.9	68.61± 0.8	21.66	6.79± 0.1
75/25	129.75± 0.8	67.5± 1.1	25.12	6.39± 0.14
50/50	109.5± 0.9	64± 1	34.43	5.55± 0.11
25/75	89.25± 1	60.5± 0.9	50.10	5.4± 0.13
12.5/87,5	78.03± 1.7	58.11± 2.2	63.25	5.04± 0.09
0/100	69± 3.1	57.4± 4.5	82.05	4.8± 0.1

Ratio WAS /OMW: mixing ratio of waste activated sludge /Olive Mill Wastewater
TS: Total solids ,VS: Volatile solids
CODt/TN: the ratio of total chemical oxygen demand /total nitrogen

Methanogenic activity test

To control the biomass composition of anaerobic co-digested waste the methane production potential of the test biomass is measured under an unlimited substrate. The acetoclastic methanogenic activity of each biomass was evaluated in shaken batch assays on the end of each kinetic. All assays were carried out in glass serum bottles (250 ml), and each biomass sample was washed with 25 mM phosphate buffer to remove any extra substrate and was dispersed by a homogenizer. The bottles containing 230 ml of 25 mM phosphate buffer were inoculated with the washed anaerobic biomass directly to a final concentration of 2 g VSS l^{-1}. The test substrates used were acetate, COD strength was set at 2000 mg COD•l^{-1}. Nutrients were not added in order to limit the biomass growth during the test period. The medium and the headspace were filled with N_2 gas at 1 atm (101 k Pa). The bottles were incubated in the dark at 55ºC. All measures other than specifically described here are given elsewhere by Pat-Espadas et al. (2015). Methane gas production was determined through the liquid (11.2% w/v KOH Solution + Thymol Blue Indicator) displacement method according to Jawed and Tare (1999) and Esposito et al. (2012). The maximum specific methanogenic acetoclastic activity (SMAA) (ml CH_4/VSS /h) was calculated from the slope of the cumulative CH_4 versus time graph.

Kinetic models

Tow models to estimate performance parameters were used. The cumulative methane production data from the experiments were fitted to the modified Gompertz equation (MGE) given by (*Eq. 1*), so this equation plot the cumulative methane production according to the time (Maamri and Amrani, 2014).

$$M = \mathrm{P.exp} \quad -\mathrm{exp}\ [\frac{R_m.e}{P}(\lambda - \mathrm{t}) + 1] \qquad \text{(Eq.1)}$$

where M is the cumulative methane production (l), P the methane production potential (l), R_m the maximum methane production rate (l d^{-1}), λ the duration of lag phase (d) and t is the duration of the assay (time) at which cumulative methane production M is calculated (d).

The Logistic equation (LGE) a model which has been used for anaerobic fermentation, as well as, for estimate the methane generated from sewage sludge (Donoso-Bravo et al., 2010). In this case, a modified version of the logistic function was used (*Eq. 2*).

$$M = \frac{P}{1 + \mathrm{exp}(4\mathrm{R}_m(\lambda - \mathrm{t})/\mathrm{P} + 2)} \qquad \text{(Eq.2)}$$

The parameters P, R_m, and λ were estimated for each of the digesters using the OriginPro8 software.

Statistical analysis

All assays were conducted in triplicate. The data on characteristics of the substrate with different mixing ratios were expressed as mean ± standard deviation. The data on performances of each digester were expressed as mean ± standard deviation during the operation period. A one-sample t-test was used to test the significance of the results and $p < 0.05$ was considered statistically significant. The statistical analysis of regression was qualified by an analysis of variance (ANOVA) and Akaike's test by OriginPro8.

Results

Identification of phenolic compounds extracted from OMW

The identification of phenolic compounds was performed by relevant molecular mass data from GC-MS. GC provided the separation of the major biophenols in the OMW extracts as illustrated in *Figure 2*. The phenolic composition of the OMW ethyl acetate extract is summarized in *Table 3*.

Anaerobic digestion

The cumulative methane production (ml) during the codigestion of WAS/OMW is shown in *Figure 3*. As was the case for different ratios of a mixture for a retention time of 32 days. Methane production started immediately from the first day of digestion in all the digesters.

Table 3. *Phenolic compounds (or related analytes) found in ethyl acetate extract of olive mill wastewater identified by GC-MS*

Fraction	Compounds	Retention time (min)	Fragments	Molecular weight	Formula	(%)
1	SuccinicAcidDimetyhlEster	2.89	55. 115	146	$C_6H_{10}O_4$	5.723
2	MethylCatechol	3.522	53.81.109	124	$C_7H_8O_2$	1.327
3	4-Ethylphenol	4.575	77. 107. 122	122	$C_8H_{10}O$	1.173
4	Vanillic acid	4.846	70 . 78. 126	168	$C_8H_8O_4$	0.455
5	Pyrocatechol	5.011	64. 110	110	$C_6H_6O_2$	1.987
6	A-Terpinolene	6.76	41 .91	136	$C_{10}H_{16}$	1.545
7	Tyrosol	7.89	41 .81.123.138	138	$C_8H_{10}O_2$	0.668
8	Vanillin	7.97	109.122.151	152	$C_8H_8O_3$	0.668
9	3,4,5 TrimethoxybenzoicAcid	9.062	39.53.93	212	$C_8H_{10}O_3$	0.040
10	Dihydroeugenol	9.29	31. 137	166	$C_{10}H_{14}O_2$	0.352
11	p-Coumaric Acid	9.916	147. 164	164	$C_9H_{12}O_4$	0.294
12	DecarboxymethylElenolicAcid	10.38	139.08	184	$C_9H_{12}O_4$	1.037
13	Hydroxytyrosol	10.694	109.137	154	$C_8H_{10}O_3$	7.227
14	Protocatechuic acid	10.87	76.107.126	154	$C_7H_6O_4$	5.955
15	3,4,5 TrimethoxybenzoicAcid	11.02	39.53.93	212	$C_8H_{10}O_3$	0.015
16	Syringic acid	12.59	155.180.182.197	198	$C_9H_6O_5$	0.038
17	4-Hydroxycinnamic acid	13.597	46.104.146.163	164	$C_9H_8O_3$	0.577
18	Gallic acid	14.371	135.150	170	$C_7H_6O_5$	0.438
19	Pinorisinol	14.90		358	$C_{20}H_{22}O_6$	0.010
20	MethylLinoledaite	15.13	41. 55. 65. 81.95	294	$C_{19}H_{34}O_2$	0.612
21	Luteolin	15.842	77. 135	285	$C_{15}H_{10}O_6$	0.042
23	DecarboxymethylLigstrosideAglycon	16.14	41.97	304	$C_{17}H_{20}O_5$	0.017
23	palmitic acid	16.446	29. 69	256	$C_{16}H_{32}O_2$	0.021
25	Dehydrodieugenol	16.66	164	326	$C_{20}H_{22}O_4$	0.002
26						
27	9-Octadecanoic Acid(Z)methyl ester	17.35	41. 55. 69. 81.97 .264	282	$C_{18}H_{34}O_2$	1.198
28	Ferrulic acid	17.569	88.118.149	194	$C_{10}H_{10}O_4$	0.008
29	Cafeic acid	19.31	89.134.151			0.018
30	Octadecanoic acid	17.79	43.69. 73.284	372	$C_{22}H_{44}O_2$	0.018
31	10-Hydroxy DecarboxymethylAglycon	17.861	336.01	336	$C_{17}H_{20}O_7$	0.017
32	Bis(2-Ethylhexyl)Phthalate	18.696	57. 149.167. 279	390	$C_{24}H_{38}O_4$	0.05371
33	Linoleic acid	19.54	139	280	$C_{18}H_{32}O_2$	0.012
34	Oleic acid	19.68	69.85	282	$C_{18}H_{34}O_2$	0.008

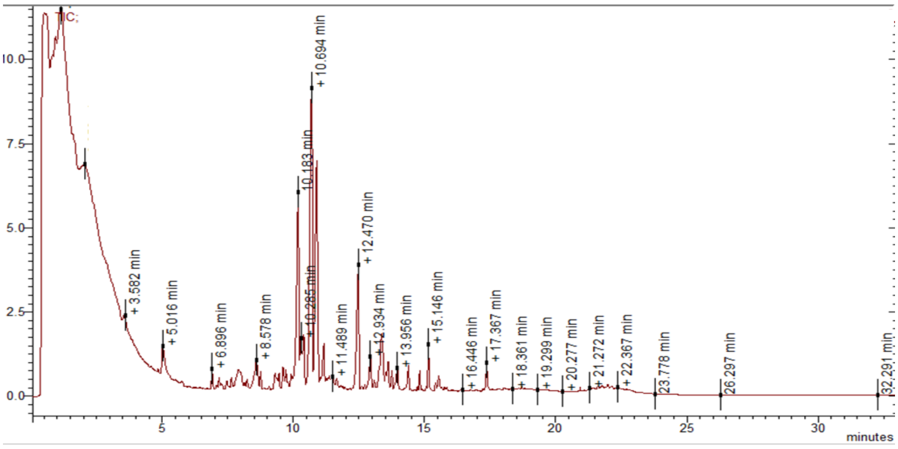

Figure 2. *GC-MS chromatograms of Phenolic monomer (or related analytes) found in ethyl acetate extract of olive mill wastewater*

The cumulative methane is better in case of codigestion than monodigestion (WAS /OMW =100/0 and 0/100), especially at the ratio 87.5 / 12.5 of WAS/OMW with the highest specific methane yield value (*Figure 3*) comparatively. The kinetic parameters of the AD process are constantly used to analyze the performance of digesters and design appropriate digesters, which are also useful to considerate inhibitory mechanisms of degradation (Kabouris et al., 2009). With an assumption that methane produced is a function of bacterial growth in batch digesters, to quantify analytically parameters of the batch growth curve, the MGF and LGF were selected to fit the cumulative methane production data. Values of parameters obtained are summarized in *Tables 4 and 5*.

It has been observed that the cumulative methane produced is well fitted with the two models as is evident from the correlation coefficient R^2 (0.9) between the experimental and predicted values along with the parameter estimated.

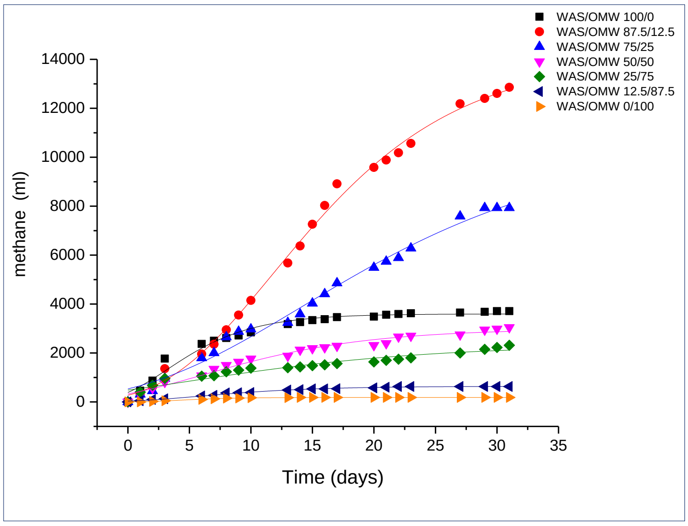

Figure 3. *Cumulative methane production at a different mixing ratio of waste activated sludge /Olive Mill Wastewater (WAS /OMW)*

Table 4. *Values of modified Gompertz equation and statistical measures for the kinetic model for Cumulative methane productions at a different waste activated sludge/Olive Mill Wastewater mixing ratio*

Ratio WAS /OMW (%)	**R^2**	**P (ml)**		**R_m (ml/day)**		**λ (day)**		**F Value**	**Prob>F**
		Value	**Stand Error**	**Value**	**Stand Error**	**Value**	**Stand Error**		
100 /0	0.97	3597.53	0.51	332.28	62.09	2.18	0.73	2048.39	0.0000
87.5 /12.5	0.99	14197.76	0.63	608.85	320.61	10.77	0.72	6651.40	
75/25	0.98	10582.33	2.47	301.16	816.69	13.14	0.20	1898.26	
50/50	0.97	2997.91	0.48	143.70	101.17	5.07	0.55	1605.77	
25/75	0.90	2348.29	1.70	76.10	227.52	4.18	0.32	553.41	
12.5/87.5	0.99	641.43	0.02	42.73	8.69	4.83	0.81	5271.44	
0/100	0.99	184.06	0.008	25.57	1.35	3.05	0.88	8718.94	

Ratio WAS /OMW: mixing ratio of waste activated sludge /Olive Mill Wastewater
P: the methane production potential
R_m: the maximum methane production rate
λ: the duration of the lag phase

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Table 5. *Values of modified logistic equation and statistical measures for the kinetic model for Cumulative methane productions at a different waste activated sludge/Olive Mill Wastewater mixing ratio*

Ratio WAS /OMW (%)	R^2	P (ml)		R_m (ml/day)		λ (day)		F Value	Prob>F
		Value	Stand Error	Value	Stand Error	Value	Stand Error		
100 /0	0.95	3555.08	1.05	456.63	71.79	3.88	0.63	1258.96	0.0000
87.5 /12.5	0.99	12856.97	1.05	968.24	266.89	13.05	0.66	3155.21	
75/25	0.97	8920.91	2.38	477.63	489.07	15.25	0.02	1243.84	
50/50	0.95	2877.57	0.78	203.73	98.22	7.81	0.36	983.20	
25/75	0.89	2272.75	1.87	101.71	199.61	8.20	0.89	465.32	
12.5/87.5	0.98	623.21	0.07	62.35	10.60	6.99	0.70	2381.88	
0/100	0.99	182.71	0.01	38.70	1.44	4.40	0.87	6752.96	

Ratio WAS /OMW: mixing ratio of waste activated sludge /Olive Mill Wastewater
P: the methane production potential
R_m: the maximum methane production rate
λ: the duration of the lag phase

The Akaike test (*Table 6*) confirms that the model of the MGF has lower AIC value and so is more likely to be correct. This model is 6139.83 times more likely to be correct.

Performance data (*Table 7)* shows that the WAS/OMW ratio of 87.5 / 12.5 favored the degradation of the organic matter, considering the best VS reduction (69.91 ±1.72). The methane yield increases slightly with the addition of OMW and remains stable until the WAS/OMW ratio of 75/25.

Table 6. *Akaike test result*

	Residual sum of squares	N° parameters	AIC	Akaike Weight
modified Gompertz equation	6031.5483	3	133.85471	0.99984
modified logistic equation	13329.2795	3	151.29981	1.63E-04

AIC: Akaike information criterion

Table 7. *Performances of mono- and co-digestion*

Ratio WAS /OMW (%)	pH_f	SMAA (ml CH_4/Gvss/h)	Specific Production (l/g VS)	CH_4 (%)	Methane yield (l/g VS_r)	VS_r (%)
100 /0	8.35 ±0.17	1.09± 0.07	0.125	70	0.629	19.94 ±2.78
87.5 /12.5	8.16±0.2	3.13 ± 0.03	0.517	71	0.74	69.91 ±1.72
75/25	7.89±0.3	1.51 ± 0.4	0.391	45	0.67	58.49 ±1.03
50/50	6.24±0.2	0.63 ± 0.06	0.117	27	0.25	46.84 ±0.83
25/75	5.89±0.16	0	0.097	18	0.18	53.90 ±1.64
12.5 /87.5	5.29±0.1	0	0.027	13	0.15	18.39 ±1.1
0/100	5±0.09	0	0.008	10	0.1	8.01 ±0.87

pH_f:final pH
SMAA: specific methanogenic acetoclastic activity
VS_r: Volatile solids reduction

We can easily observe that the increase in the amount of OMW in the mixture gives a peak correlation (*Figure 4*) in the methane production parameters. Then the SMAA also gives a peak profile.

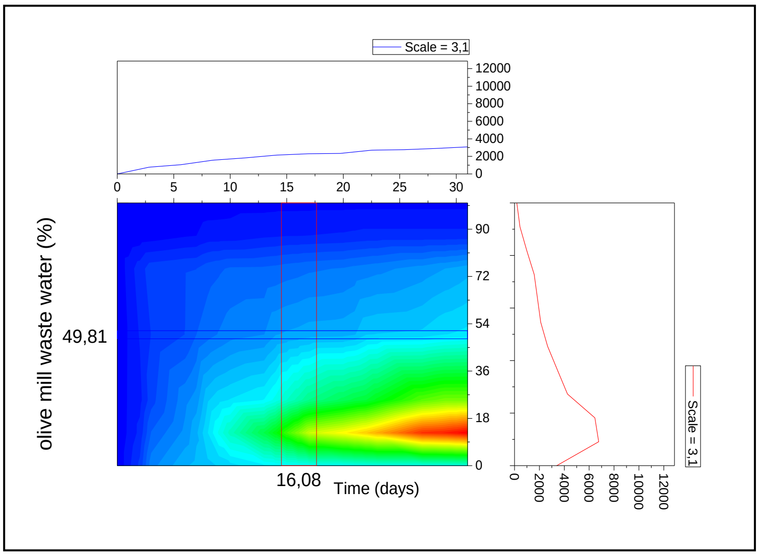

Figure 4. *Correlation between cumulative methane production and Olive Mill Wastewater amount and retention time*

In light of these results, the investigate the WAS/ OMW optimum mixture ratio by the modelling of these correlations (*Figure 5*) via the Gauss amplitude equation (*Eq. 3*) has given a good statistical significance (*Tables 8 and 9*).

$$Y = Y_0 + Ae^{-\frac{(X-Xc)^2}{2w^2}} \qquad \text{(Eq.3)}$$

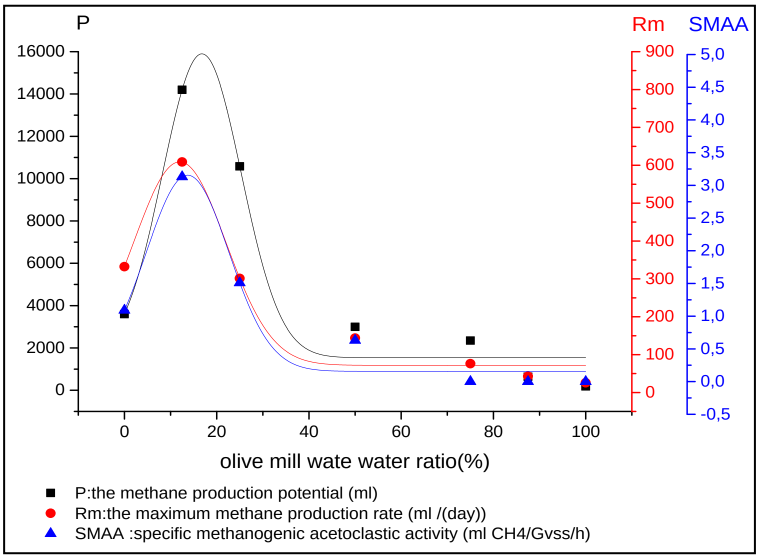

Figure 5. *Gauss amplitude correlation between Olive Mill Wastewater ratio and the methane production potential, the maximum methane production rate, and specific methanogenic acetoclastic activity*

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Table 8. *Values of Gauss amplitude function for the correlation between Olive Mill Wastewater amount and the methane production potential, the maximum methane production rate and Specific methanogenic acetoclastic activity*

	R^2	Y_0		xc		w		A		FWHM	Area
		Value	SE	Value	SE	Value	SE	Value	SE	Value	Value
P	0.93	1537.03	672.9	16.79	1.04	8.54	1.5	14360.36	1814.2	20.11	307413.4
R_m	0.93	71.77	26.06	12	1.19	9.98	1.2	537.33	58.15	23.50	13443.7
SMAA	0.92	0.15	0.15	13.69	1.25	8.96	1.1	2.99	0.35	21.11	67.41

Y_0; xc; w ; A; FWHM : Gauss amplitude function parametres
S E: Standard Error

Table 9. *ANOVA analysis of regression*

	DF	Sum of Squares	MeanSquare	F Value	Prob>F
P	4	3.36048E8	8.4012E7	46.64	0.005
R_m	4	592609.95	148152.48	54.76	0.004
SMAA	4	13.36	3.341	33.76	0.008

P: the methane production potential
R_m: the maximum methane production rate
SMAA: specific methanogenic acetoclastic activity

Based on the adjusted correlation coefficient ($R^2 > 0.9$), we can approve a good agreement and advocates greater significance of the model.(Niladevi et al., 2009). ANOVA of the fitted model for the P, R_m, and SMAA (*Table 9*) demonstrates that the model is significant due to the F-value of 46.64, 54.76, 33.76 respectively and the low probability P-value ($p \leq 00$). Generally, an F-value with a low probability P-value suggests a significant regression model (Rene et al., 2007). The maximum value of P, R_m and SMAA are obtained from the (x,y) coordinates of amplitude, and that the Limit of synergistic effect is calculated from the addition of Xc to the W value, *Table 10* summarizes these results.

Table 10. *Optimization parameters*

Parametres	Maximum value	Olive mill wastewater ratio %	
		Optimum ratio	Limit of the synergistic effect
P (ml)	15897.39	16.79	25.33
R_m (ml /d)	609.1	12	21.98
SMAA (ml CH_4/VSS/h)	3.14	13.69	22.65

P: the methane production potential
R_m: the maximum methane production rate
SMAA: specific methanogenic acetoclastic activity

To verify the limited mixing ratio, by considering the waste activate sludge (WAS) as the main compound and the olive mill wastewater (OMW) as the additional mixing compound in this study, the calculation of the relative fraction “*f*” from the specific production of the co-digestion dived to the WAS mono-digestion alone was done. This factor give an exponential correlation (*Figure 6*) with good R^2 (0.962) and statistical significance ($p = 0.016$). Logically to obtain synergistic effect the “*f* “must be upper than1 ($f > 1$).This condition is verified when the Olive mill wastewater ratio is lower than 22 (%).This result validate the result presumed by the Gauss amplitude model 21.98 and 22.65 (%) predicted by R_m and *SMAA* data, respectively.

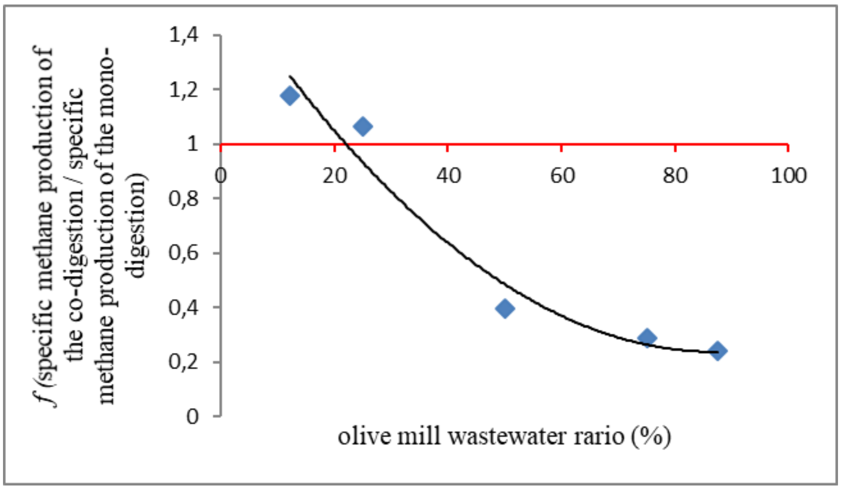

Figure 6. *Exponential correlation between Olive mill wastewater ratio and factor “f” of increase in the specific methane production*

Discussion

The GC-MS identification of phenolic compounds present in olive mill wastewater showed qualitative differences amongst the different research paper according to cultivars and their geographical origin (La Cara et al., 2012; Leouifoudi et al., 2014).

The best cumulative methane production was done at WAS/OMW ratio of 87.5/12.5 (12000 ml) without adjusted pH (neutral), this result is comparable to the result of codigestion of olive mill wastewater and swine manure established by Azaizeh and Jadoun (2010) with a 14000 ml of biogas under adjusted pH (neutral), at 38 ± 2°C for 11 days using Gadot sludge (25 g) or Prigat sludge (25 g) added to 250 ml of olive will waste water (Azaizeh and Jadoun, 2010). In our study the addition of OMW (WAS/OMW: 87.5/12.5) up to 72.34% in the cumulative production, this result is better comparativly to the codigestion of pig manure and OMW at pig/OMW ration equal to 60/40 which up to 40% the production of both substrates (Kougias et al., 2010).

The synergistic effect for co-digestion of waste activated sludge and olive mill wastewater at 87.5/12.5 mixture ratio was mostly attributed to a greater extent of volatile solids reduction and higher specific methane yield. This is a result of a supplementary requirement of nutrients and micro/trace elements from co-substrates, as the catalytic centers of the involved enzymes in methanogenic pathways (Pagés-Díaz et al., 2014; Xie et al., 2017). The olive mill wastewater is an additional source of Ni metal which is implicated in three recognized pathways of methanogenesis. This last one is regularly metal-rich enzymatic pathways when Fe is the most abundant metal, followed by Ni and Co, and smaller amounts of Mo (and/or W) and Zn. Fe is primarily present as Fe–S clusters used for electron transport and/or catalysis. Ni is either bound to Fe–S clusters or in the center of a porphyrin unique to methanogens, cofactor F430. Zn occurs as a single structural atom in several enzymes (Glass and Orphan, 2012). However, other origins of synergisms must additionally be considered, such as the optimization of the C/N ratio (Xie et al., 2017).

However, OMW alone and other mixtures have acidic pH and low biogas yield because the methanogenic bacteria are most efficient at pH 6.5–8 (Mao et al., 2017). Based on the pH value at the end of digestion (*Table.7*) the WAS/OMW ratios: 75/25 and 50/50 reinforce the system buffer capacity.

The obtained results show clearly that SMAA and the lag phase λ values are moderately varied with initial condition COD/ N, pH, TS, VS, and waste type. This can be done in the dynamic of biomass composition and to the selective synergistic effect of bacterial communities. Li et al. (2015) in their study of AD system, of cattle and/or swine manure by metagenomics assays, noted that the substrate type, the ratio of co-substrate, play major roles in the COD/N ratio of substrate and free ammonia which play a central factors in the development and structuring of the bacterial communities in AD systems.

This peak profile of correlation can be explained by the limitation of the AD at high amounts of OMW % in the medium of fermentation. Though the WAS microorganisms have a limited capacity to degrade the high molecular-mass polyphenols in OMW biotreatment (Sayadi et al., 2000) and the inhibition of AD of OMW imply polyphenolic compound and the long chain fatty acids, which are considered as a toxic compound in the system of the AD (Hamdi, 1996; Hernandez and Edyvean, 2008; Saha et al., 2011; Oz and Uzun, 2015; Al-Mallahi et al., 2016). According to Borja et al. (1997) the cinnamic, benzoic, caffeic and protocatechuic acids are an inhibitor of acetoclastic methanogenesis at a limit concentration.

The long-chain fatty acids present in OMW are also responsible for its toxicity to methanogenic bacteria (Hamdi, 1992). The oleic acid is present in high concentration in OMW (Sayadi et al., 2000; Visioli and Galli, 2002; Elkacmi et al., 2017) which gives a high concentration of oleates. Comparatively to Sousa et al. (2009) an oleate added have given a stoichiometric value considering complete oleate oxidation. This indicates that acetoclastic activity was affected by oleate, so methane production in these cultures could be justified just by hydrogenotrophic activity or a limited acetoclastic activity (Sousa et al., 2013). Referring to Wu et al. (2017) the improvement in methane production rate was limited to the oleic acid concentration.

Conclusion

These results are consistent with the batch conducted tests, where the best performance was observed through a clear peak correlation which describe that the optimum settings for the maximum value of methane yield and acetoclastic activity are delimited by OMW components. The lower performance degrees achieved for a high OMW amount in codigestion. Gauss amplitude function is a good model to predict the area limits of the microbial communities synergistic effect which are not able to avoid inhibitory effects associated with the inhibitors present in OMW. Further research studies are needed to determine the microbial and biochemical properties of substrates. In addition, a follow-up study on the effects of individually isolated inhibitors on process performance.

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