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Comparative Evaluation of Biogas Yield and Physicochemical Properties of Three-Phase and Traditional Olive Oil Mill Wastes – The Most Suitable Choice for Efficient Anaerobic Digestion

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ABSTRACT

Olive oil mill waste is characterized by its high organic matter content, especially fatty acids, polyphenols, sugars, and proteins. These nutrients can be used as a source of energy for biogas production. However, olive oil mill waste can also contain heavy metals such as lead, cadmium, copper, and zinc that can be absorbed by plants. In addition, very high concentrations of heavy metals can also inhibit the anaerobic digestion process by affecting the methanogenic bacteria involved in biogas production The aim of this research is to determine the composition of solid and liquid rejections from traditional and continuous three-phase crushing systems, by analyzing samples from different oil mills in the eastern region of Morocco. We also applied the technology of anaerobic digestion of solid and liquid waste forms of oil mills, to make a link between the biogas yield and the physicochemical characteristics of these wastes. The results suggest that traditional oil mill wastewater (Discontinuous OMWW) has high organic matter, nutrients, and heavy metals content and a low concentration of phenolic compounds, which can increase its biogas production potential with a production of 10.02 Nml/g VS, while three-phase wastewater (Continuous OMWW) has limited biogas production potential (3.83 Nml/g VS) due to the low organic matter and nutrients content, and high concentration of phenolic compounds. Three-phase olive pomace (Continuous OMSW) has a higher biogas production (9.28 Nml/ g VS) than traditional olive pomace (Discontinuous OMSW) with 5.91 Nml/g VS. In fact, the lower content of phenolic compounds and volatile fatty acids favors their anaerobic digestion and improves their biogas production. In conclusion, the selection of the type of waste adapted for biogas production must be based on the physicochemical and microbiological characteristics of these wastes.

Keywords: olive oil mill wastes, biogas yield, physicochemical characteristics, discontinuous waste, continuous waste.

INTRODUCTION

World's olive oil industry has grown rapidly in recent years due to the increase in the number of extraction units and the appearance of new methods of crushing olives, with an annual production of approximately 3,215,000 tons (International Olive Council, 2022). The majority of production (about 2,500,000 tons) comes mainly from countries in Southern Europe, the Middle East, and North Africa (Alburquerque et al., 2004). According to projections for the 2020–2021 campaign, Morocco produces about 140,000 tons of olive oil, of which the eastern region contributes one of the largest production areas, this production is mainly concentrated in the province of Taourirt.

Olive oils are extracted by several methods, either traditionally (discontinuous system) by applying hydraulic pressure, or moderately (continuous system) by using rotary hammers to crush the olives (ben Sassi et al., 2006). However, two types of residues are generated during the extraction of olive oil, whose volume and physicochemical characteristics depend on the olive oil extraction process (el Yamani et al., 2019). One solid lignocellulosic called olive pomace (OP), its main components are cellulose, hemicellulose, and lignin, making these by-products suitable for thermal utilization, with an average lower calorific value of 19.167 kcal/kg (Mata-Sánchez et al., 2013), its current price is 80–100 €/t in Andalucía (Spain) (Cuevas et al., 2019). Regarding the elemental composition, the concentrations of chlorine and copper vary from 90 mg/kg to 435 mg/kg and from 0.6 mg/kg to 2.3 mg/kg, respectively (García Martín et al., 2020). With a percentage of 0.1 to 0.2% of potassium (K), 0.03 to 0.06% of phosphorus (P), and 0.2 to 1.146% of total phenols. The other residue, called oil mill wastewater (OMWW), is a red-to-black liquid waste with high electrical conductivity (6,000–16,000 µs) and an acidic PH (4.0–5.5), with high values for most pollution parameters BOD5 (40–95 g/l); COD (50–180 g/l); LD50 toxicity for fish (8.7%). Its main components are phenolic compounds, sugars, and organic acids (Dermeche et al., 2013). OMWW contains other elements, in particular, potassium (2.37–10.8 g/l), zinc (12–19.62 mg/l), phosphorus (0.3–1.5 g/l), ferric, copper, and magnesium (Vlyssides et al., 2004).

The bacterial structure and diversity in OMW are highly affected by harvesting and cultivation practices and the specific olive variety from which the OMW is generated. Based on several previous studies, (Spyridon et al., 2013) concluded that olive oil mill effluents are specifically dominated by Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Firmicutes, and Actinobacteria, with about 20% of coliforms. In addition, high levels of Staphylococcus spp. have been identified in olive oil mill waste. Concerning fungal diversity, the population of yeasts and molds is very present in the waste of olive oil mills, known by their tolerance to phenolic compounds, and their capacity to reduce phenolic compounds and sugars (Spyridon et al., 2013).

Oil mill wastes have negative effects on soil microbial populations, plant growth and germination, aquatic fauna, and river ecosystems, even on-air quality (el Yamani et al., 2019; Rincón et al., 2008). Anaerobic digestion (AD) has been identified as a promising solution for the treatment of olive oil mill waste (Messineo et al., 2020). Which allows at the same time the stabilization of the waste and the recovery of energy in the form of biogas. In recent years, research in the field of anaerobic digestion has intensified mainly due to the gradual depletion of fossil fuels, as well as concerns about increasing greenhouse gas emissions (Pellera et al., 2016).

The aim of this research is to determine the composition of solid and liquid rejections from traditional and continuous three-phase crushing systems, by analyzing samples from different oil mills in the eastern region of Morocco. We also applied the technology of anaerobic digestion of solid and liquid waste forms of oil mills, to make a link between the biogas yield and the physicochemical characteristics of these wastes.

MATERIALS AND METHODS

Sampling environment and method

The OMWW and OP samples used in this study were collected from the different mills (continuous and discontinuous systems) and mixed to obtain a representative sample for each system, during the olive oil processing season from November 2021 to March 2022 in the eastern region of Morocco (Taourirt province), the liquid samples were collected in sterile bottles and the solid samples in sterile sachets and transported in ice boxes. A part of our samples was used directly for microbiological analysis, the other part was kept at -16°C for physicochemical analysis. Before the physicochemical analyses, the solid waste (OMSW) was crushed and diluted to obtain a liquid sample.

Chemical analyses

In order to characterize the physicochemical parameters, the following parameters were measured in triplicate. The PH of the samples was determined using a PH meter WTW 197i, and the electrical conductivity (EC) by a conductivity meter WTW 190i, expressed in ms.cm-1. Ammonium (NH_4^+) by indophenol blue method (APHA, 2017). Total phenolic compounds (after centrifugation and filtration) were quantified by spectrophotometry according to the Folin-Ciocalteu method (De Marco et al., 2007). The Chemical Oxygen Demand (COD) was measured by using the potassium dichromate method, and the

Biological Oxygen Demand (BOD5) was determined by incubation in a BOD Meter at 20°C for 5 days (ISO 15705, 2002; NF EN 1899-2, 1988)

According to Rodier (Rodier J et al., 2009), chloride was determined by the titrimetric method of Mohr with silver nitrate and potassium chromates. Sulfates by turbidimetric method 4500-$SO4^{2}$- E (APHA, 2017). Phosphates by colorimetric method with ammonium molybdate (Murphy and Riley, 1962). Suspended solids (SS), Total solids (TS), Volatile solids (VS), volatile fatty acids (VFA), and alkalinity were analyzed according to APHA standard methods (APHA, 2017)

Metal analysis

The determination of metals was performed by inductively coupled plasma spectrometry (ICP) after acid digestion of the samples performed according to the method described by (Elabdouni et al., 2020), All analyses were performed in duplicate. The metals selected for this study were cadmium (Cd), zinc (Zn), lead (Pb), copper (Cu), iron (Fe), manganese (Mn), potassium (K), and magnesium (Mg).

Microbiological analyses

Microbiological analyses included the counting of total aerobic mesophilic flora (TAMF), total and thermotolerant coliforms (TC and TtC), staphylococci, yeast, and molds. However, in general, the method involves taking a representative sample of the waste, diluting it, and then seeding known portions of these dilutions on a selective culture medium containing specific substrates for the growth of these microorganisms. Colonies are then counted after incubation at an appropriate temperature and the number of colonies is expressed in CFU (Colony Forming Units) per gram (Zaier et al., 2017).

Anaerobic digestion

The process of anaerobic digestion was initiated by introducing the inoculum (activated) and substrates into 30cl plastic bottles in appropriate amounts and then adding sterile water to bring the total volume to about 300 ml. The eight reactors were sealed and incubated in a warm room at 35°C with manual agitation (Pellera and Gidarakos, 2016). The inoculum used in this study consisted of a cattle manure sample collected from a laboratory-scale mesophilic anaerobic reactor. The TS and SV contents and PH of the inoculum were 24%, 17.37%, and 7.10, respectively. The composition of the reactors is presented in Table 1. We used the 75:25% substrate/inoculum ratio (Rubio et al., 2019). Each test was repeated a second time, as shown in the experimental setup diagram (Figure 1).

Table 1. Experimental protocol for preparing the eight digesters

Reactors	Substrates	Substrate/Inoculum ratio (g VS)	Substrate (g)	Inoculum (g)	PH
T1	OPM	12.2/4.07	21.60	23.44	7
T2	OMM	12.2/4.07	213.43	23.44	7
T3	OPT	12.2/4.07	18.39	23.44	7
T4	OMT	12.2/4.07	78.39	23.44	7

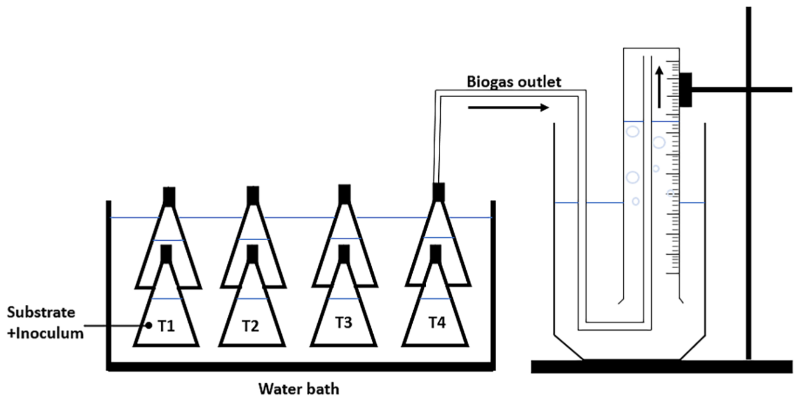

Figure 1. Experimental setup diagram

The quantity of biogas generated by the substrate is obtained by the continuous liquid displacement method (Figure 1). And then Mesophilic biogas volumes were corrected to normal liters (0 °C to 1 atmosphere) (Afilal et al., 2013), and then dividing it by the initially added amounts of substrate VS.

RESULTS AND DISCUSSION

The analyzed solid and liquid residues of the several olive oil extraction processes presented brown to black color discharges, the slightly acidic of about 5 for the four types of analyzed waste, which is in accordance with the literature (Gunay and Karadag, 2015; Messineo et al., 2020; Oz and Uzun, 2015). The OMWW is characterized by a high electrical conductivity (about 10.29 µs/cm), due in particular to the high concentration of dissolved mineral salts, and also to a large amount of Na Cl added to the olives for preservation purposes in the plant (Table 2). We noticed a low concentration of total and suspended solids in the OMWW from the continuous extraction systems compared to those from the discontinuous system, thus less colloidal solids which have a low settle ability (El-Gohary et al., 2009). Compared to OMWW, OMSW has higher contents of total solids and volatile solids (66.48%; 56.64%) approved by many studies (Dermeche et al., 2013).

OMW is very rich in organic matter, expressed in terms of BOD_5 (biological oxygen demand) and COD (chemical oxygen demand) (Oz and Uzun, 2015). Our results show very high COD and DBO values for traditional OMSW (329.70 g O_2/l; 73.00 g O_2/l) followed by traditional OMWW (128 g O_2/l; 25.00 g O_2/l) and then three-phase OMSW (94.40 g O_2/l; 24.00 g O_2/l) and three-phase OMWW (39.75 g O_2/l; 19.00 g O_2/l) (Table 2). From these results, we concluded that OMW from discontinuous extraction processes is richer in organic matter, thus a higher pollution potential which is confirmed by many studies (ben Sassi et al., 2006; Vlyssides et al., 2004).

However, the concentration of total phenolic compounds in the studied OMW is low compared to what is found in other studies (Oz and Uzun, 2015; Pinto-Ibieta et al., 2016). The highest concentration of phenols was observed for OMWW from continuous treatment units with 5.57 mg/ml (Table 2). These differences in phenol contents could be due to the crushing method used, the olive variety, the cultivation method, and also the ripening degree of the olives (ben Sassi et al., 2006).

Olive mill waste has a significant amount of VFA and a moderate quantity of TAC that can be recovered for biogas production and reduce the environmental impact of this waste. Our results suggest that OMWW from the traditional process contains a lower concentration of VFA and TAC (on average 22 g acetic acid/l and 7 g $CaCO_3$/l, respectively), compared to those produced by the three-phase process (32.5 g acetic acid/l; 12 g $CaCO_3$/l, respectively). On the other hand, OMSW produced by the three-phase process contains concentrations of VFA and TAC in the range of 5.9 g acetic acid/l; 11 g $CaCO_3$/l, which is lower compared to OMSW produced by the traditional process (Table 2).

Phosphate, nitrogen, potassium, calcium, magnesium, chlorine, and sulfur are necessary substrates for the development of anaerobic bacteria (Afilal et al., 2014). In terms of elemental composition, the four waste groups analyzed generally had high concentrations of ammonia nitrogen (NH_4^+), phosphate, sulfate, chloride, potassium (K), magnesium (Mg), and copper (Cu). The solid residues (OMSW) in this study have very high nutrient concentrations compared to the liquid residues (OMWW) (Table 1). In addition, the residues from discontinuous extraction systems are more loaded with these elements compared to continuous extraction systems, which is confirmed by many studies (García Martín et al., 2020; Vlyssides et al., 2004). Some metallic trace elements were also determined for the four types of residues, such as iron, cadmium, zinc, lead, and manganese. They are present at low concentrations (Table 2). In overview, the physicochemical characteristics including phenolic compounds, nutrients, VFA, and TAC concentrations varied considerably depending on the type of waste and the olive oil production process used.

The results of counting indicate the total absence of bacteria indicators of fecal contamination and Staphylococcus, and this can be due mainly to the antimicrobial activity of phenols present in our waste and the acidic PH (Belén et al., 2022), but also to the methods of sample collection. In this study, the samples were collected directly after the crushing of olives, which explains somewhat the absence of these bacteria. For total aerobic microorganisms or total aerobic mesophilic flora (TAMF), values of 2.4102; 8.5102; 4.8 104;

Table 2. Chemical characteristics of OMWW and OMSW from two olive oil extraction processes

Parameters	Discontinuous process (Traditional)		Continuous process (Three-phase extraction system)	
	OMSW	OMWW	OMSW	OMWW
pH	5.30	5.45	5.72	5.10
Electrical conductivity (ms/cm)	7.52	10.49	7.27	10.22
Total phenols (mg/ml)	2.97	2.92	2.76	5.57
COD (g/l)	329.70	128.00	94.40	39.75
BOD_5 (g/l)	73.00	25.00	24.00	19.00
Total solids (TS) (%)	67.45	20.5	58.5	6.00
Volatile solids (VS) (%)	66.48	15.6	56.64	5.73
Total suspended solids (g/l)	__	32.610	__	18.491
Total phosphate (mg/l)	11.00	1.70	8.20	1.50
Total sulfate (mg/l)	79.46	75.21	65.32	50.30
NH_4^+ (mg/l)	24.00	10.00	17.50	8.50
Chlorure (mg/l)	5550.00	1449.00	3502.50	1320.00
VFA (g acetic acid/l)	14.2	22	5.9	32.5
Alkalinity (g $CaCO_3$/l)	19.4	7	11	12
Cd (mg/l)	0.218	0.212	0.212	0.212
Cu (mg/l)	0.212	0.241	0.266	0.230
Fe (mg/l)	0.383	0.079	0.063	0.026
K (mg/l)	140.5	36.46	48.51	20.11
Mg (mg/l)	9.649	1.848	3.005	1.50
Mn (mg/l)	0.098	0.024	0.049	0.01
Pb (mg/l)	0.160	0.097	0.106	0.069
Zn (mg/l)	0.212	0.077	0.209	0.020

and 2.2 105 CFU/g were obtained for the four types of samples, the wastes with the lowest total aerobic microorganisms are the wastes with higher concentrations of phenolic compounds, which confirms the antimicrobial activity of the phenols present in the olive crushing wastes (Dermeche et al., 2013). Yeasts and molds are known for their ability to grow in olive oil production waste, due to their tolerance to phenolic compounds (Spyridon et al., 2013). In our study values of the order of 8.4 104; 2.5 102; 4 101; and 5.1 103 CFU/g were found (Table 3).

Estimation of biogas potential

After 21 days of incubation of the reactors containing the four types of waste studied in a warm room at a temperature of 35°C, the biogas production kinetics (Figure 2) shows that the wastewater from traditional olive oil mills (Discontinuous OMWW) produces the highest amount of biogas with a production of 10, 02 Nml/g VS, followed by solid waste from three-phase crushing units (Continuous OMSW) with a production of 9.28 Nml/g VS, solid waste from traditional crushing

Table 3. Microbiological characteristics of OMWW and OMSW from two olive oil extraction processes

Parameters	Discontinuous process (Traditional)		Continuous process (Three-phase extraction system)	
	OMSW	OMWW	OMSW	OMWW
Thermotolerant coliforms (CFU/g)	<10	<10	<10	<10
Total coliforms (CFU/g)	<10	<10	<10	<10
Total aerobic microorganisms (CFU/g)	$8.5\ 10^2$	$4.8\ 10^4$	$2.2\ 10^5$	$2.4\ 10^2$
Coagulase-positive Staphylococci (CFU/g)	$<10^2$	$<10^2$	$<10^2$	$<10^2$
Yeasts and molds (CFU/g)	$2.5\ 10^2$	$5.1\ 10^3$	$8.4\ 10^4$	$4\ 10^1$

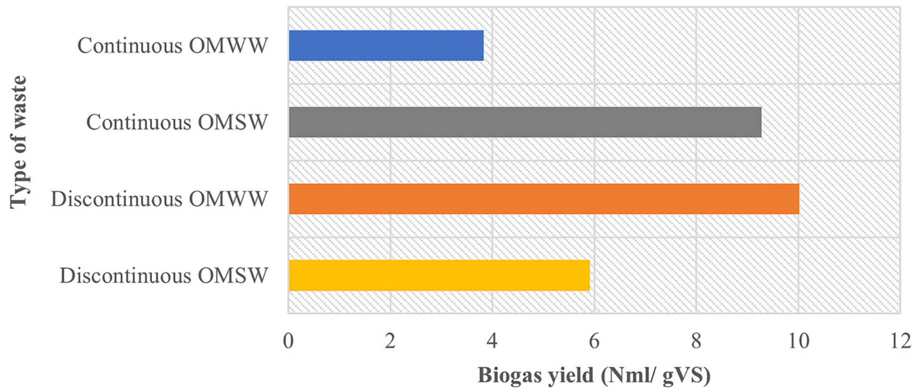

Figure 2. Biogas yield of the four studied wastes

units (Discontinuous OMSW) with 5.91 Nml/g VS, and finally wastewater from three-phase olive oil mills (Continuous OMWW) produce the lowest amount of biogas 3.83 Nml/g VS.

This difference in biogas production can be explained by the chemical compositions of these wastes, in which continuous OMWW contains very high concentrations of phenolic compounds, volatile fatty acids, and very low levels of organic matter and nutrients compared to the other types of waste studied. While the discontinuous OMWW is richer in organic matter and nutrients and poor in phenolic compounds, which explains the significant production of biogas. However, it is important to note that our results may differ from several studies, this difference may be due to the variety of olive, climatic conditions, processing conditions, and anaerobic digestion used, as well as the characteristics of these residues.

Regarding the production of biogas by OMSW, the results of anaerobic digestion demonstrated that the production of biogas was higher for three-phase olive pomace, with an average biogas production of 9.28 Nml/g SV, compared to traditional olive pomace, which produced on average 5. 91 Nml/g SV, can be explained by the low content of three-phase OMSW in phenolic compounds and volatile fatty acids. According to our research, there are no studies in the literature that have specifically compared biogas production between traditional olive oil mill wastes and those produced by the three-phase process.

The biogas production versus time graph (Figure 3) shows that biogas production fluctuates, with maximum production at the beginning of the anaerobic digestion, it may indicate a variation of the substrate characteristics, such as its content of organic matter, nitrogen, phosphorus, fats, proteins, and sugars. In general, microorganisms tend to degrade first the easily biodegradable organic matter present in the substrates, such as sugars, amino acids, short-chain fatty acids, and similar compounds. Then the bacteria start to degrade more complex and resistant organic compounds such as proteins, long-chain fats, and polysaccharides. the second stage of degradation

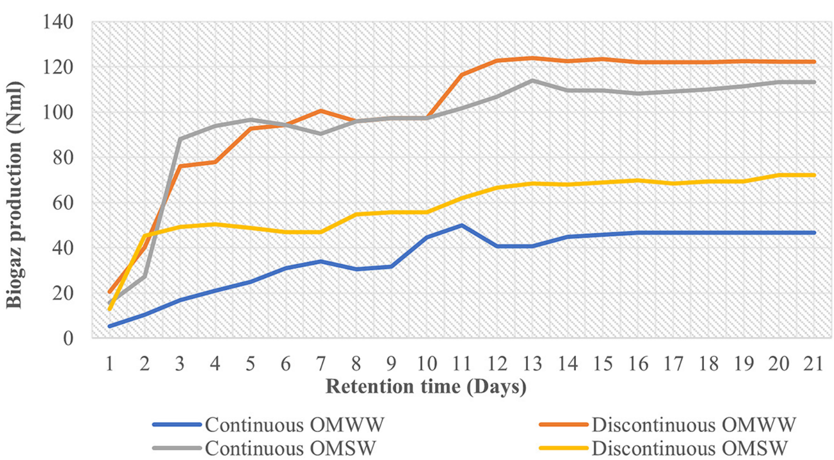

Figure 3. Graph of biogas production versus time for the four studied wastes

may take longer and require the participation of a more diverse and specialized community of microorganisms. many studies have examined this variation in substrate characteristics during anaerobic digestion and have confirmed these results (Borja et al., 2002; Rincón et al., 2008; Al Afif et al., 2019).

We noticed that the production of biogas is limited for the four types of residues, this can be attributed to the presence of compounds that can limit the anaerobic digestion, such as acid PH, volatile fatty acids (VFA) an excess of VFA can cause an inhibition of the growth of methanogenic microorganisms responsible for the biogas production. And polyphenols, have a toxic effect on methanogenic microorganisms, which reduces biogas production. Proven by numerous studies (Gannoun et al., 2007; Fezzani and Ben Cheikh, 2009).

CONCLUSION

Olive oil mill waste is an important source of organic matter and nutrients for biogas production. Traditional and three-phase waste have different physicochemical and microbiological characteristics, which influence the production of biogas from each type of waste. According to this study, wastewater from traditional olive oil mills and olive pomace from three-phase olive oil mills are more adapted to anaerobic digestion. The use of these residues for biogas production is a sustainable energy alternative that contributes to the reduction of fossil fuel dependency and environmental protection, but strategies must be implemented to optimize biogas production from each waste type.

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