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ORIGINAL PAPER

Effects of Oxidative Treatments on Biomethane Potential of Solid Olive Residues

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Abstract

As energy systems transition toward renewable resources, anaerobic digestion (AD) is actually receiving growing attention. AD relies on biochemical methane potential (BMP) tests to determine the methane potential of by-products of carbonious nature. This investigation aims to understand how an oxidative treatment, like the Fenton reaction, influences the BMP, starting from solid residues of olive oil production, coming from the two-phase extraction systems (TPES). We compared two different olive pomaces (with and without stones), both from TPES. The Fenton treatment here proposed is able to produce three effects in the employed matrices: improving the speed of BMP decreasing the bacteriostatic effect of phenols, reducing the H_2S content in the produced biogas (precipitating it as FeS) and enhancing the production of methane in the first four weeks of the test.

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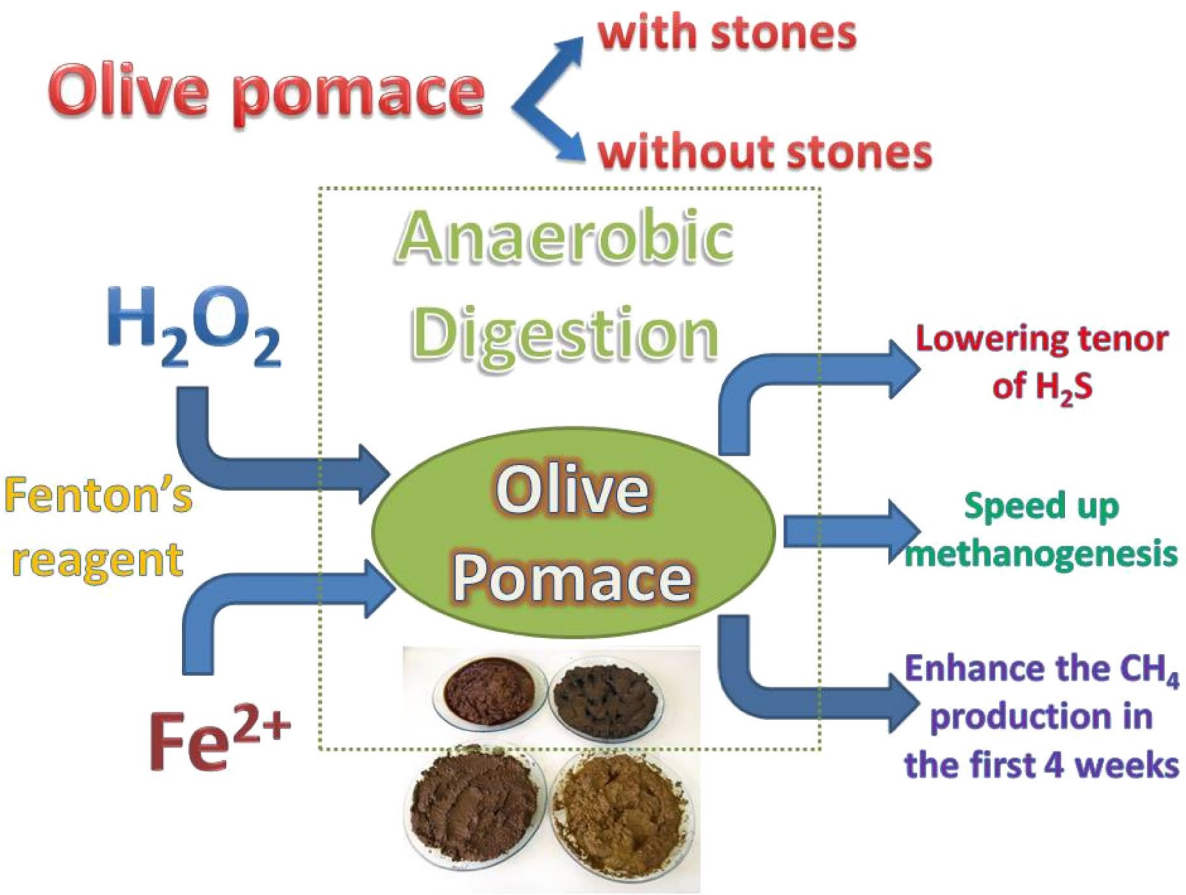
Graphical Abstract



Keywords Olive pomace · Biomethane · Anaerobic digestion · Fenton reaction · Polyphenols · Biomass valorisation

Statement of Novelty

This study aims to solve the problem related to semi-solid by-products of the modern olive oil industry. At present, the wet olive pomaces (WOP) represent one of the most relevant environmental impacts connected to the olive oil production. The major obstacle to the exploitation of the residual biomass is due to the high phenols content presents in it which hinders the use of one of the most promising technologies for the management of organic matter, the anaerobic digestion (AD). Several research contributions were performed to valorise the olive residues but, to the best of our knowledge, few of them have accomplished an oxidative treatment based on Fenton's reagent directly on the solid phase. Our study, allowing prompt biomethane production, mitigates the bacteriostatic effect of phenols and, at the same time, restricts the presence of H_2S in the biogas.

Introduction

During the Seventies, the traditional discontinuous process for olive oil extraction, in which the ground paste is subject to pressure by use of pressing mats to expel the liquid content (olive oil and vegetation water), was replaced by a new continuous extraction system based on the use of metal crusher and horizontal centrifugal machines known as "decanters". The traditional extraction technology is now outdated for the low capacity to process the olive fruits and for the greater manpower needed, even if the produced olive pomace (OP) might represent an additional income for the miller because it is suitable to be addressed to the industrial recovery of residual oil [1, 2].

The technological evolution of extraction systems, based on crushers and decanters, has the side effect of a reduction of value of the solid by-products due to the higher moist content and the lower quantity of oil in the OP (Table 1) [3]. In the Nineties, with the spread of two-phase extraction

Table 1 Olive pomace characteristics according to the olive oil extraction system (Source: Di Giovacchino [3])

Constituents	Olive oil extraction system			
	Pressure	Three-phase	Two-phase and a half *	Two-phase
Pomace quantity (kg/t olive)	250–350	450–550	550–650	800–850
Water (%)	22–35	45–55	55–62	65–75
Residual oil on fresh weight (%)	6–8	3.5–4.5	3.5–4.5	3–4
Fibre (%)	20–35	15–25	12–20	10–15
Stone (%)	30–45	20–28	15–20	12–18
Ash (%)	3–4	2–4	3–4	3–4
N (mg/100 g)	250–350	200–300	200–300	250–300
P (mg/100 g)	40–60	30–40	35–45	40–50
K (mg/100 g)	150–200	100–150	100–180	150–250
Phenols (mg/100 g)	200–300	200–300	250–350	400–600

*A three-phase centrifugal decanter with a low water consumption

technology, at least in the main production Countries (*e.g.*, Spain), the wet olive pomace (WOP) has become an environmental issue, especially in the area of Mediterranean basin, where the majority of the world olive productions occurs [4].

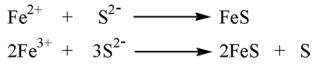
Recent evolutions in olive oil processing have brought to the exploitation of solid by-products with the development of systems capable to recovery the stone fragments and to carry out an optional second extraction of residual oil [5]. This has conducted to a different type of solid by-product, a pomace with higher percentage of pulp and fibre and with lower content in lignin: the destoned wet olive pomace (DWOP). In addition, the olive pit fragments can be addressed to both: energy purpose as fuel [5–7] and raw material for the production of chemicals (*e.g.,* furfural) [8]. The olive stone can also be separated from the olive pulp before the decanter-step for the niche production of high-quality olive oil [9].

The solid by-products coming from the three-phase decanter or from the three-phase centrifugal system with low consumption of water ("two-phase and a half") can be still positively considered for the owners of oil plants since, despite the absence of any income due to the very low residual oil content, the OP is easily movable to other plants able to valorise it. On the other hand, it is no longer convenient, for the factories, when the OP comes from two-phase extraction systems (TPES) since it would be necessary a particularly expensive thermal treatment to reduce the high humidity content [3, 7, 10].

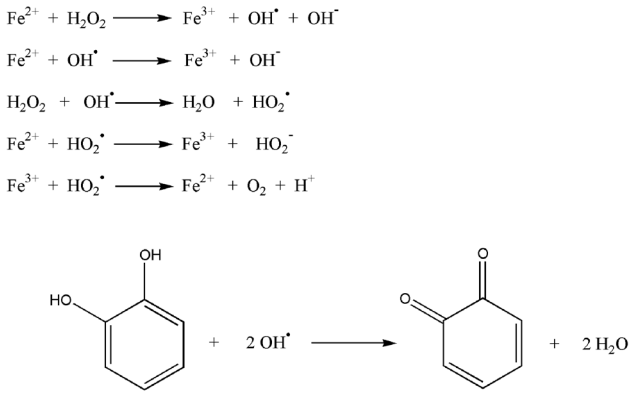
Regarding the two-phase OP, to overcome the related environmental impacts and to better valorise it, some innovative uses have been proposed in the past [11]. Worthy of note are several applications such as soil conditioner [12, 13], livestock feed [14] and building material [15]. Then OP can actually be considered also as a raw material for valuable organic compounds (*e.g*., pectin, antioxidants) [16] or a renewable energy resource [17]. Even if the heterogeneity in the phenolic compound distribution represents an obstacle [18], such by-products of the olive oil industry can be equally considered an inexpensive source of antioxidants, suitable for the production of bioactive compounds, addressing them to the production of nutraceuticals [19] and as added inside foods, highly requested by the consumers [20, 21]. On the other hand, additional parts of olive trees can be used as a continuous source of these valuable compounds during the year; in fact, olive iridoids are present in high concentration also in the leaves [22, 23].

Concerning the exploitation of OP for energetic purposes, some inconveniences may arise such as the caking inside the fuel handling plants [24]. In spite of the multiple potential uses, the profitability of the innovative plants for the exploitation of the olive solid residues is unsure nowadays and only a small part of the worldwide produced pomace is processed [18, 19, 24].

Among the sustainable approaches to be considered in the near future, biological transformations of the water-rich OP could be an easy-to-apply, cheap and profitable choice [25–27]. One of the most promising techniques is the anaerobic digestion (AD) [28–30]. It is a core technology in the sustainable management of organic matter [31]. Several authors agree that, for moist olive wastes, the AD is preferable, from an environmental point of view, to the OP oil extraction [32] or to the conventional disposal on soil [33]. On the other hand, one of the main drawbacks is related to the high content of phenols [34]. These compounds possess a bacteriostatic and phytotoxic effect and can significantly contribute to the alteration of the surrounding ecosystems [35] when freely released into the environment. It also should be noted that this class of compounds is only partially degraded during AD; in fact, in the methanogenic phase there is a partial abatement of phenols but in the acidogenic conditions, they remain unchanged [36]. As a solution to this problem, a pretreatment, that provides to overcome the



Scheme 1 Reactions of Fe salts with H_2S in anaerobic digester liquor



Scheme 2 Fenton's reaction and interaction of $OH^{\cdot}$ with phenolic moieties

bacteriostatic effect of phenolic fraction, so improving the biogas production, can be proposed. Most of the treatments to increase the biomethanation, which do not use physical methods, employ alkaline derivatives, eventually in synergic action with an oxidant, like hydrogen peroxide [37–41] although Fe^{2+}/Fe^{3+} salts are normally added to the organic feed or directly to the anaerobic digester for the in situ reduction of the biologically produced H_2S [42, 43] (Scheme 1), however relatively few literature reports deal with combined treatments on OP using soluble Fe salts in association with H_2O_2 (Fenton reagent) despite to its well-known ability to oxidize any phenolic substance [44–48].

Usually, the Fe salts added to the biomass, before the insertion into the digester, have no impact on the AD process [49]. On the other hand, Fe^{2+} combined with H_2O_2, produces $OH^{\cdot}$ [50], able to fully oxidize the phenol compounds, even though by a non-selective reaction. This contributes to remove their adverse effects on the biomethanation [51, 52] (Scheme 2).

The present study will show how an oxidizing treatment, like the Fenton's reaction, can impact the formation of methane in the biomethanation reaction (by measuring the Biochemical Methane Potential, BMP) carried out on two different types of pomaces (WOP and DWOP), both deriving from TPES. In this context, also the influence of Fe salts on H_2S production will be assessed. The goal of our study is to propose a realistic oxidizing pretreatment of WOP and DWOP, able to be introduced in the olive agro-industrial sector, minimizing the additional costs. We provide evidence that the presence of Fe/H_2O_2 system allowed overcoming the bacteriostatic effect of phenols speeding up the developing biogas and improving the quality of biomethane thanks to the reduction of the H_2S content.

Materials and Methods

Materials

Iron (II) chloride tetrahydrate (ReagentPlus®, 98%), hydrogen peroxide (35%), sulphur standard for ICP OES (1000 mg/L, in water), sodium carbonate (powder, ≥ 99.5%, ACS reagent), Folin-Cicolteau's reagent, ethanol (96%, EMSURE® Reag. Ph Eur), gallic acid (97.5–102.5%) and nitric acid (ACS reagent, 70%) were purchased from Merck KGaA (Darmstadt, Germany). Deionized water was produced by a Christ Ministil P6 apparatus.

Inoculum

An active inoculum was collected from a biogas plant that digests cattle manure provided by Azienda Agricola Bruni, Sutri (VT), Italy. The particulate matter (> 1 mm), consisting of large fibrous materials (*e.g.*, straw), was removed by passing the digestate through a sieve. The latter fraction was degassed in mesophilic conditions (35–38 °C) for 10 days before using it in the experiments [53].

Substrate and Pretreatments

The WOP and DWOP were provided by an olive oil mill located in Abruzzo region (Tiberio Ernesto s.a.s., Tollo, Chieti, Italy). The BMP were measured comparing the untreated raw material with the pretreated one. To pretreat the WOP and DWOP samples, a fixed concentration of $FeCl_2$, corresponding to 4.2 g of Fe/kg of fresh OP, was added. This amount is commonly used in small and medium size AD plants to reduce the H_2S content inside biogas [54]. In the case of untreated WOP and DWOP, rather than the Fe^{2+} solution, deionized water was added in the amount of 255 mL/kg of OP.

WOP experiments were conducted as follows: 230 mL of 0, 4.4 or 8.9 M H_2O_2 solutions containing, each one, 13.5 g of $FeCl_2{\cdot}4H_2O$, were uniformly sprinkled on 900 g of WOP, previously spread out on a watch glass (with diameter 50 cm). After a careful kneading step (the oxidation reaction is strongly exothermic), made by a stainless-steel spatula, the dough was left to stand overnight and hence it was stored in a suitable container ready to be used for the biomethanation experiments (Fig. 1). DWOP experiments were conducted in the same conditions, but considering four different H_2O_2 concentrations: 0, 1.8, 3.5 and 7.1 M.

The experiments were executed in triplicate, when the OP was present in enough amounts, or in duplicate when the DWOP was not sufficient.

Analytical Determinations

The total solid content (TS), the volatile solid content (VS), and pH were measured according to APHA methods [55]. The lipid fraction was quantified by the Randall method using a dedicated apparatus (SOXHTRACTION; VELP Scientifica) [56]. Briefly, the extraction was made by mixing the OP samples in boiling *n*-hexane, followed by a washing step with cold *n*-hexane. After that, the defatted matrices were used to evaluate the fibre content by sequential extractions using a FIBRAMATIC PBI apparatus according to the Van Soest method [57].

The elemental analyses were performed on mineralized OP samples using an Agilent® MP-AES spectrometer (microwave-plasma atomic emission spectroscopy); 0.5 g of dried (105 °C, 24 h) and finely powdered pomace (WOP or DWOP) were added to a PTFE vessel where, subsequently, 6 mL of 65% HNO_3 and 2 mL of 30% H_2O_2 were added. The vessels were capped, transferred to the microwave reaction chamber (START D—Microwave Digestion System of Milestone S.r.l.) and microwaved at 500 W (heating at 200 °C, followed from maintenance at 200 °C for 15 min, then decreasing temperature until 110 °C and keeping it for others 15 min) at a pressure of 45 bar. After the samples had cooled, they were transferred to 100 mL polypropylene tubes and made up to a volume of 50 mL with ultrapure water (18.3 MΩ) (Zeneer Power II, Human Corporation) [58].

Fig. 1 Example of OP samples (WOP) untreated (top, left) and treated samples (only with Fe, on the top, on the right side; with Fe and 4.4 M H_2O_2, on the bottom, on the left side; with Fe and 8.9 M H_2O_2, on the bottom, on the right side). Note the slight whitening observed on the treated sample with the higher concentration of H_2O_2

Total phenol contents were evaluated by the Folin-Ciocalteu's method [59] and, for the quantitative data, a calibration curve was built by using gallic acid at six known concentrations (0.1, 0.2, 0.3, 0.4, 0.5 and 1 g/L), in ethanol solutions (the equation of a straight line is: $Y = 1.1933X$; $R^2 = 0.99833$), in the same experimental conditions. However, while measures conducted directly on pomaces alone (both, WOP and DWOP) gave reproducible total phenol content values, also in accord with published data [3, 60], treated pomace samples (with Fe and H_2O_2) had overestimated values. This is the reason why we retained that the real and correct total phenol values were that obtained with the samples not containing Fe; so, we measured the total phenol content in Fe-containing pomaces only for comparative purpose, extrapolating the relative percentage of phenol abatement. Basically, we normalized the total phenol values using, as normalizing factor, the value obtained analysing the Fe-containing pomace without H_2O_2. We obtained the following values (as % of phenol abatement): for WOP, 27.8% (4.4 M) and 54.4% (8.9 M); for DWOP 8.4% (1.8 M), 38,9% (3.5 M) and 53,2% (7.1 M).

The total sulphur content was measured by an ICP apparatus (Varian 720-ES Series) prior acidification of the liquid samples with concentrated HNO_3. The resulting solutions were then collected in 10 mL volumetric flasks with ultrapure water and then analysed by ICP instrument. Concentrations of samples were adjusted with HNO_3 2% v/v in order to be within the concentration range of the calibration straight, that was built in the 0.1–100 µg/L range, starting from S standard solution of 100 µg/L. Measurements were carried out with a wavelength of 182 nm.

Experimental Setup

To measure the BMPs, we used an Automatic Methane Potential Test System II (AMPTS II) provided by Bioprocess Control Sweden AB. The apparatus showed high reproducibility in our tests, with a relative standard deviation below 8% for each experiment after two weeks of measurements, typically under 2% for the whole set of experiments. The BMP assay was performed using the AMPTS II (BPC Instruments, Sweden) equipped with 15 test vessels (500 mL) equipped with agitators (mixer on/off time 50/40 s, mixer speed adjustment 80%); a thermostatic water bath (18 L) was used to keep at 37 °C the vessels [61] (Fig. 2). The working biomass for the BPM assay was set to 400 g while the inoculum to substrate ratio was chosen based on the VS values; for WOP tests the ratio was 0.4 whereas for DWOP tests the ratio was 0.5 [39, 62]. Blank assays, conducted in triplicate, and in presence of

Fig. 2 AD used for the experiments

the same amount of inoculum (400 g), were also performed for evaluating the residual BMP of the inoculum and calculating the effective methane production in each substrate. An aliquot of each OP was further treated with Fe^{2+} alone, in order to assess the role of the added salt to the BMP coming from the H_2O_2-free experiments. In terms of Fe concentration, we used the same order of magnitude as those reported in previous biomethanation studies without H_2O_2 (see above for the amount; "Inoculum") [42, 43].

Before starting with experiments, the entire apparatus was purged by a N_2 flux for 5 min to achieve the anaerobic conditions. The biogas current, that might contain both CO_2 and H_2S, was passed through an 80 mL vial filled with a 3 M NaOH solution, containing a few drops of thymolphthalein (to keep the pH under control). The resulting CH_4 gas current was addressed to gas volume measuring device. The effective BMP was calculated as follows:

$$CH_4 net = CH_{4\,test\ vessel} - CH_{4\,Blank}\frac{gVS_{inoculum\ in\ test\ vessel}}{gVS_{inocolum\ in\ blank\ vessel}}$$

Results and Discussion

Chemical characterizations of both WOP and DWOP were carried out and data are shown in Table 2. As expected, the lignin content value was higher in WOP samples. All the other obtained data were in accord with previous literature reports [1].

Table 2 Main characteristics of WOP and DWOP

Composition	WOP	DWOP
Water content (%)		
Weight loss at 105 °C	66.56 ± 0.32	75.15 ± 0.21
Oil content (%)		
Residual oil	8.26 ± 0.34	10.46 ± 0.13
Residual oil (fresh weight)	2.70 ± 0.15	2.60 ± 0.05
Fibre content (dry weight) (%)		
Sugars and proteins	18.88 ± 0.45	27.96 ± 0.87
Hemicellulose	18.86 ± 0.19	13.68 ± 0.84
Cellulose	16.69 ± 0.45	13.56 ± 0.85
Lignin + cutin	37.30 ± 0.75	34.31 ± 2.56
Acid-Insoluble Ash	0.03 ± 0.01	0.02 ± 0.002
Acidic/alkaline conditions (pH unit)		
pH	4.72 ± 0.03	4.92 ± 0.02
Phenol content (mg/kg)		
Total phenols	2967 ± 82	4361 ± 95
Elemental composition (dry weight) (mg/kg)		
K	25,320 ± 150	20,260 ± 50
Na	560 ± 5	570 ± 6
Fe	239 ± 4.5	233 ± 7.2
Mn	1.0 ± 0.02	1.0 ± 0.05
Zn	13.8 ± 0.47	11.3 ± 0.45
Cu	3.3 ± 0.08	6.5 ± 0.04

The production of methane was followed for 55 days, continuously measuring its volumetric amount. Results are illustrated in Fig. 3a (WOP) and Fig. 4a (DWOP), where the entire amount of produced methane was reported, while in Figs. 3b and 4b the daily production of methane was shown. The diversity between the WOP and DWOP (i.e. in phenol and fibre contents) involve to use a bit different experimental conditions in order to assess the most suitable one to improve the BMP yields. Therefore, otherwise from WOP test, in the DWOP experiments, an additional oxidative treatment at low concentration of H_2O_2 (1.8 M) was considered. This was carried out using a duplicate test for entries 8, 10 and 11 in Table 3.

In both experiment typologies, when the sample produced a quantity of biomethane lower, compared to the vessel filled with only the inoculum (blank assay), a negative value was reported in the BMP test graph. The error bars in Figs. 3, 4 and 5 count as two standard deviations (SD). So we can consider statistically significant ($p \leq 0.05$) the differences between the obtained values, when the standard deviation error bars in the graphs do not overlap.

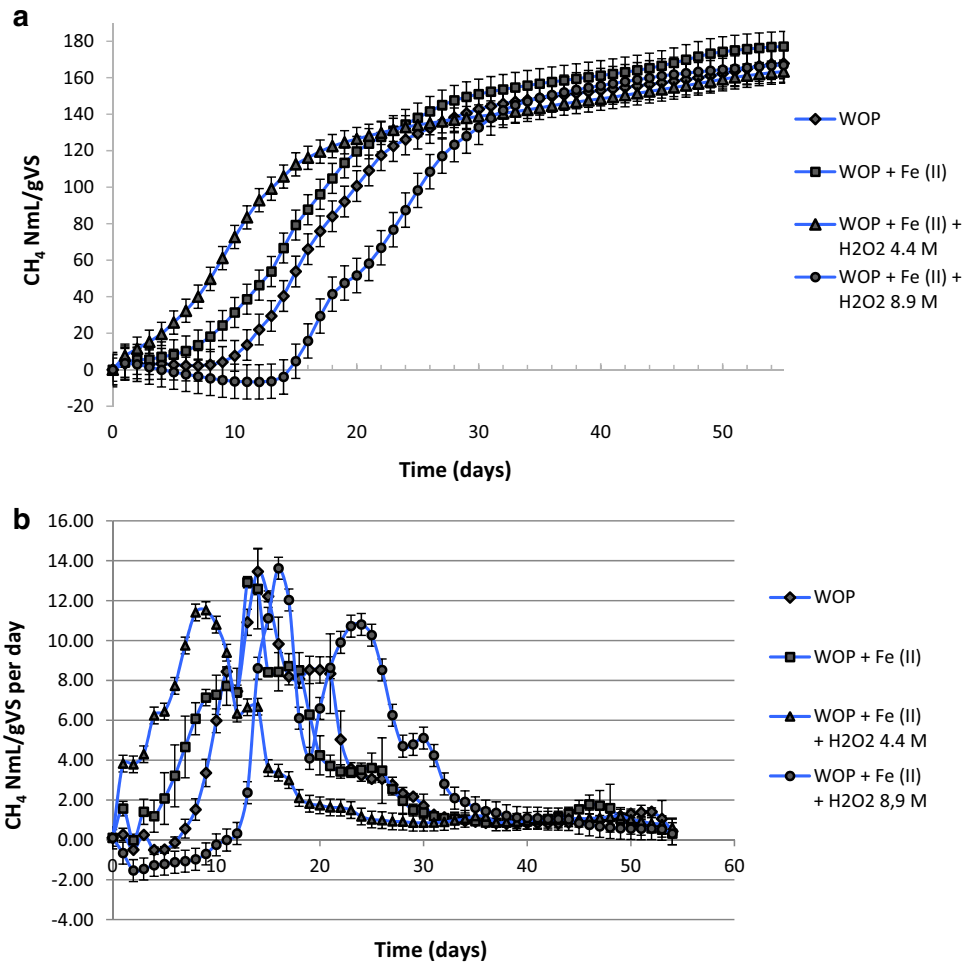


Fig. 3 **a** Cumulative CH_4 production (NmL/gVS) in WOP tests. The error bars in the graph represent 2 SD. **b** Daily CH_4 production (NmL/gVS per day) in WOP tests (55 days). The error bars in the graph represent 2 SD

WOP Experiment Results

As shown in Fig. 3b, from the untreated WOP (entry 2, Table 3) the CH_4 production started from day 8 with the maximum daily production (13.46 NmL/gVS) appearing during the 14th day. The treated with Fe^{2+} and 4.4 M H_2O_2 WOP (entry 4, Table 3) showed an intense production of methane almost immediately, with the maximum daily production (> 11 NmL/gVS) around the 8–9th days. The treated with only Fe^{2+} WOP produced, in the first 20 days of the test, an intermediate amount of CH_4 between the values from untreated and the treated with 4.4 M H_2O_2/Fe^{2+}samples. In entry 5 (with the highest amount of H_2O_2: 8.9 M) the CH_4 production started form the 15th day and displayed three peaks of production during, respectively, the 16th (13.62 NmL/gVS), the 24th (10.80 NmL/gVS) and the 30th day (5.1 NmL/gVS). At the end of the experiments, biomethane was produced in a total amount ranging from 163 to 167 NmL/gVS, with the only exception of entry 3, which produced a slightly higher value (177.1 NmL/gVS) (see Fig. 3a).

DWOP Experiment Results

In the DWOP experiments, the biomethane production was comparable during all the time with maximum daily production, for all the treatments, around the 20th day (Fig. 4b). Only in the experiment conducted with the highest amount of H_2O_2 (entry 11, Table 3; 7.1 M of H_2O_2), the CH_4 production started with a delay of 3–4 days, at least compared with the other experiments. In this case, the chart curve of the daily methane production was different, evidencing three maximum peaks, respectively after 18, 22 and 27 days.

At the end of the test (after 55 days) the best performance in terms of biomethane production was achieved by entry 7 (the untreated DWOP sample; 233.6 NmL/gVS) followed by entry 9 (experiment with 1.8 M of H_2O_2; 225.4 NmL/gVS), then entries 8 and 10 (both 215 NmL/gVS) and finally entry 11, that showed the lowest methane production value (191.4 NmL/gVS).

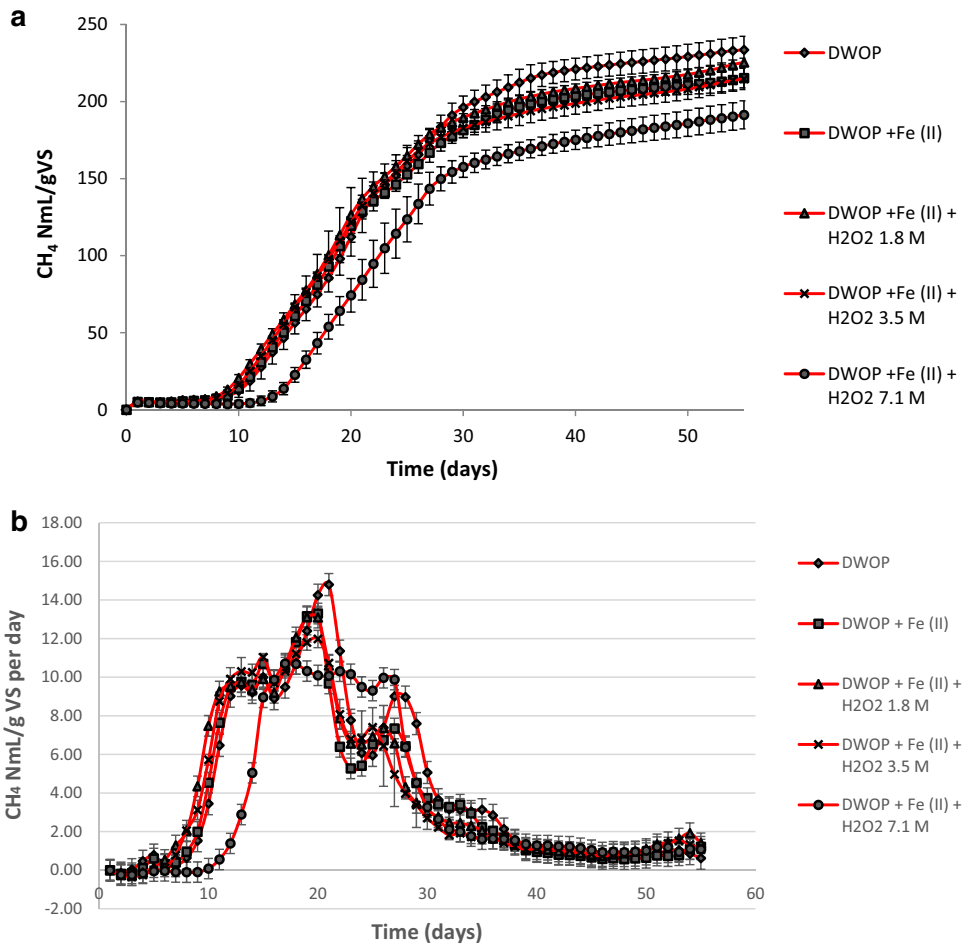


Fig. 4 **a** Cumulative CH_4 production in DWOP tests (55 days). The error bars in the graph represent 2 SD. **b** Daily CH_4 production (NmL/gVS per day) in DWOP test. The error bars in the graph represent 2 SD

Table 3 Legend for entries

Entry	Type of experiment
1	Inoculum WOP Test
2	WOP
3	WOP + $FeCl_2$
4	WOP + $FeCl_2$ + H_2O_2 4.4 M
5	WOP + $FeCl_2$ + H_2O_2 8.9 M
6	Inoculum DWOP Test
7	DWOP
8	DWOP + $FeCl_2$
9	DWOP + $FeCl_2$ + H_2O_2 1.8 M
10	DWOP + $FeCl_2$ + H_2O_2 3.5 M
11	DWOP + $FeCl_2$ + H_2O_2 7.1 M

Overview of BMP Experiments

Since the BMP experiments are particularly time consuming (55 days), we found it useful to give a half-way report (*i.e.*, until 28 days): both mildest oxidative treatments (4.4 M for WOP and 1.8 M for DWOP) were more successful compared with the untreated samples and the samples which had undergone the most severe treatments (8.9 M for WOP and 7.1 M for DWOP) (Fig. 5). In fact, at the beginning of the tests, CH_4 was produced with a higher rate in experiments 4 and 9, but, unfortunately, after about 20 days, the production rates became equivalent, probably this can be due to the depletion of carbon source (see also Figs. 3a and 4a).

In both OPs with the highest concentrations of H_2O_2 (always in presence of Fe^{2+}), an inhibitory effect was observed, mainly at early days. These findings could be easily interpreted by the residual presence of H_2O_2 that could have an inhibitory effect on the methanogenic bacteria. Also, the intense acidification, that occurred when iron and H_2O_2 were added, could be considered a drawback for methane production; however, the buffering effect by the inoculum was to mitigate the negative influence on bacteria (see Table 4).

In Table 5, also the sulphur contents are reported (in mg/L on the alkaline trapping solution): it should be noted that both entries, 1 and 6, represent the sulphur content inside the used inoculum while the other values, namely entries 2–5 and 7–11, were obtained by subtracting, to

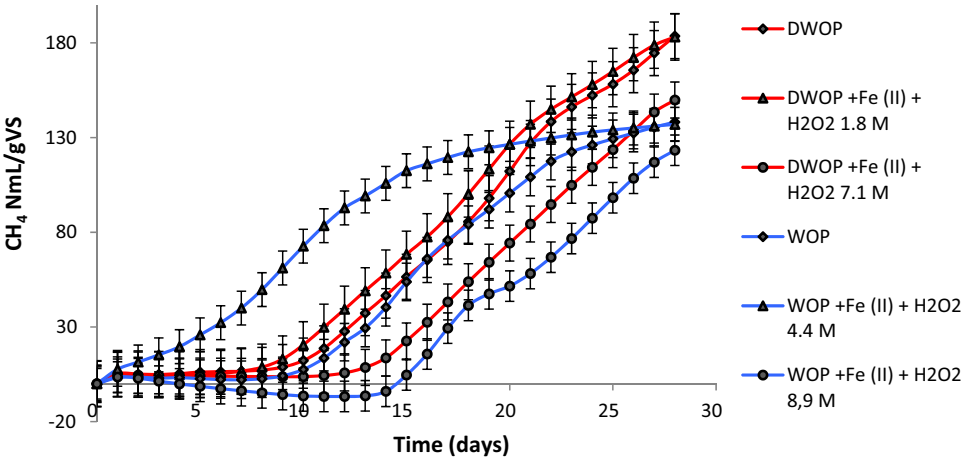


Fig. 5 Cumulative CH_4 production (WOP and DWOP) in the first 28 days (only the strongest and the mildest oxidative tests compared with the untreated matrix). The error bars in the graph represent 2 SD

Table 4 pH in WOP and DWOP test

Entries	pH of the samples[a]	pH of batch at the beginning[b]	pH of batch at the end[c]
1	7.55	7.55	8.00
2	4.72	7.22	7.83
3	3.33	7.04	7.73
4	2.98	6.91	7.68
5	2.75	6.95	7.75
6	7.78	7.78	7.93
7	4.92	7.38	7.66
8	3.41	7.10	7.62
9	3.00	7.03	7.53
10	2.83	6.85	7.56
11	2.65	6.80	7.48

[a]pH of inoculum (entries 1 and 6) and pH of both WOP and DWOP samples (entries 2–5 and 7–11)

[b]pH of inoculum (entries 1 and 6) and pH the mixtures inoculum + OP sample (entries 2–5 and 7–11): beginning of the experiment of biomethanation

[c]pH of inoculum (entries 1 and 6) and pH the mixtures inoculum + OP sample (entries 2–5 and 7–11): end of the experiment of biomethanation

the obtained values, the sulphur content of inoculum (for WOP samples, the sulphur content reported in entry 1 was used while for DWOP samples, the sulphur content reported in entry 6 was used). In this context, it should be stressed that the added iron, other than as the reagent for the generation of $OH^{\cdot}$ (Fenton reagent), also acted as sulphur sequester since it is able to precipitate H_2S as an insoluble salt like FeS [49]. The negative values of sulphur content in Table 5 suggest that both, the sulphur contained in OP and the sulphur contained inside the inoculum, were sequestered by iron and consequently, the resulting corrected values could be lower than the sulphur content inside inoculum alone (*i.e.*, without Fe).

Since the BMPs values were referred to the VS weight unit (Table 4), we can compare these values without any other data manipulations. DWOP produced methane in an amount higher than WOP (Fig. 3a *vs* Fig. 4a; 55 days, about 35% higher in DWOP samples). Apparently, the presence of stone fragments in WOP partially inhibited the biomethanation. This is not unusual since lignin often represents an obstacle, reasonably for two reasons: lignin itself, present in greater quantities in WOP, is not easily digested by microorganisms; furthermore, the release of readily biodegradable materials from lignin clusters, can occur only after an efficient disaggregation step that can contribute to make AD more efficient [63]. Among the delignification procedures, a pretreatment can be necessary and several techniques have been proposed in the past: physical, chemical, physicochemical, biological or a combination of them [64]. In our study, the physical removal of a part of lignin fraction was made by the olive oil miller, furnishing us the DWOP fraction. However, this is not the conclusive solution and a further pretreatment, which involves the chemical inertization of phenols, can be of help in the optimization of the AD from OP.

Discussion

The olive oil industry sector in Europe, with a harvest of 12.6 million of tons of olive to be used exclusively for the olive oil production (mean values, years 2016–2020) [4, 65], potentially can produce on average 2.5 million of tons of olive stone (about 20% of the whole fruit [66]) and, if processed exclusively by a TPES, considering the data reported in Table 1 [3], has the potentiality to produce approximately 10.4 million of tons of OP. Taking into consideration our data and using a hydraulic retention time of 20 days [67], we can suppose a potential production in the EU area of 335 million of Nm^3 of CH_4 per year, that

Table 5 Physical and chemical parameters of entries **1–11**

Entry	Hum. %	TS %	VS%	(VS*100)/TS	Matter in 500 mL glass bottle (g)		VS in 500 mL glass bottle (g)		VS_{inoc}/VS_{matrix}	S* (mg/L)
					Inoc	OP	Inoc	OP		
1	92.95 ± 0.02	7.05 ± 0.02	4.53 ± 0.03	64.26 ± 0.56	400.05	–	18.12	–	–	23.6^{++}
2	66.56 ± 0.32	33.44 ± 0.32	32.06 ± 0.33	95.87 ± 1.92	295.58	104.40	13.39	33.47	0.400	22.9
3	68.12 ± 0.66	31.88 ± 0.66	30.02 ± 0.83	94.16 ± 4.55	290.44	109.53	13.16	32.88	0.400	2.8
4	65.43 ± 0.57	34.57 ± 0.57	32.71 ± 0.61	94.63 ± 3.34	297.14	102.90	13.46	33.66	0.400	− 11.5
5	67.53 ± 0.18	32.47 ± 0.18	30.58 ± 0.23	94.18 ± .22	291.91	108.31	13.22	33.12	0.399	− 4.0
6	93.13 ± 0.06	6.87 ± 0.06	4.44 ± 0.12	64.62 ± 2.30	400.02	–	17.76	–	–	31.4^{++}
7	75.15 ± 0.21	24.85 ± 0.21	23.28 ± 0.21	93.66 ± 1.63	289.56	110.51	12.86	25.73	0.500	9.2
8‡	73.07 ± 0.28	26.93 ± 0.28	25.19 ± 0.31	93.53 ± 2.11	295.72	104.59	13.13	26.34	0.498	− 21.5
9	75.81 ± 0.18	24.19 ± 0.18	22.57 ± 0.18	93.29 ± 1.45	287.07	113.50	12.75	25.62	0.498	− 21.6
10‡	75.91 ± 0.28	24.09 ± 0.28	22.36 ± 0.34	92.84 ± 2.50	292.26	109.08	12.98	25.48	0.509	− 19.8
11‡	74.44 ± 0.28	25.56 ± 0.28	23.66 ± 0.32	92.57 ± 2.25	290.86	112.46	12.91	26.61	0.486	− 18.7

*S: Sulphur content; with the exception of entries 1 and 6 (see below, the ++ marker), the other values were obtained subtracting the sulphur inoculum content (for major details, see experimental)

++Inoculum sulphur content: real values, without any manipulation

‡Tests performed in duplicates

may be increased to 420 million of Nm^3 of CH_4 per year (+ 25.6%) if the OP is treated by the conditions used in entry 4.

The olive stones, with their high heating value, which ranges between 18.8 MJ/kg and 20.9 MJ/kg, can be commercialized (approximately 80–100 €/t [68]) and so it can be considered an income source for the olive miller, independently if such residues come from a two- or three-phase olive oil extraction system.

The results provide evidence that the proposed oxidative treatments can contribute to reduce/eliminate the environmentally hazardous H_2S in the biogas, with a beneficial effect on the ecosystems; moreover, it should counteract the bacteriostatic effect due to the presence of phenols. In this regard, although in our experiments the biomethane yields with Fenton treatment were not much higher than the analogue yields obtained with the untreated samples, it should be highlighted that methane was produced from treated samples already from the early days of the experiment, so contributing to optimize the reactor usage (a particularly important finding, when considering experimental trials that lasting more than 50 days). A phenol-free OP could be introduced to the anaerobic digester, according to the limits of organic load rate of the plant, and the production of biomethane should start in the first days, without a latent period, caused probably by limiting effects of the present polyphenols. Thanks to the Fenton pretreatment, the plant could be used in a continuous procedure. Furthermore, a phenol-free DWOP could be the best matrix from the olive oil industry to feed an anaerobic digester due to its highest production of biomethane per gram of VS.

Fast production of CH_4 from both, pretreated WOP and DWOP, could be positive for olive oil mills. The combined heat and power, that can be generated inside small-medium size AD plants, could help the olive oil miller to reduce the energy demand from outside, after all it was evidenced in the European Community strategy for waste-to-energy [69].

In our opinion, the extractive pathway for olive oil food processing, based on the two-phase decanter, coupled with an olive pit separator is particularly remarkable. The decanter equipped with two outlets (oil and WOP), if compared with the three-phase horizontal centrifuges, presents a lower energy and water consumption (usually called "ecological" [70]) and, as above mentioned, the olive stone fragments can be recovered in a more efficient way [7]. The oxidative treatments proposed by us could be a reliable tool to allow an optimization of energy recovery from the olive residues, making, at the end, circular the life of OP: it is transformed in a soil conditioner (the digestate; [33]) and renewable energy (methane). In addition, the suggested oxidative treatments have the potential to be used for all different kinds of OPs to be addressed to recovery energy purpose, even for small and medium industrial contexts typically of Italy and some other Mediterranean Countries; nevertheless, even when a second extraction of oil from OP and the recovery of the olive pit fragments are performed, in large olive mills [5], the Fenton treatment still be useful to valorise the residual biomass, closing the "loop" of organic material without generating wastes.

From the economical point of view, the additional cost due to the oxidative treatment can be ascribed to the H_2O_2 since iron is normally added in small and medium

size digester to upgrade the quality of biogas. The application of our idea to a real plant does not imply major plant upheavals. One possibility is to equip the plant with simple technological devices like a storage tank for the reagents, equipped with a dosing pump and a solid phase mixer, necessary to homogenize iron, H_2O_2 and OP, before introducing the dough inside the digester.

Conclusions

The sequence of OP production by a kernel separator in the olive oil extraction process, together with an oxidative pretreatment aiming to reduce the side-effect of phenols in the biomethanation, could be an efficient way for the valorisation and exploitation of the entire organic matter derived from the olive oil industry [71].

The most intriguing result regarding the BMP, for both OPs, is that DWOP showed better values (BMP at 55 days of DWOP samples are about 30% higher than the analogue values of WOP) and we retain that this is due to the higher amount of easily biodegradable organic material (higher amount of degradable sugars and lower amounts of lignin) in DWOP.

The mildest oxidative treatments proposed in our study provided evidence of promoting the methanogenesis performance until the 28th day of test, reducing or removing the bacteriostatic effects of phenols. Fenton's reagent combines two major effects: the removal of bacteriostatic phenols and the upgrading of biogas quality (diminishing the H_2S). The proposed treatment scheme has the potential to eliminate the environmental impacts associated with the olive oil industry and permits a full exploitation of the whole biomass that comes from the olive oil food processing.

The drop of pH after the treatment with the Fe^{2+}/H_2O_2 must be considered in a full-scale application of this oxidative treatment. Furthermore, we did not observe any clear role of $OH^{\cdot}$ in the disruption of lignin clusters since, as above depicted, the strongest oxidizing systems did not increase the biodegradable organic matter (methane yields did not change) [63]. In conclusion, to design a full-scale application, it will be necessary to identify the most appropriate conditions about the type of oxidative treatment to be employed, analysing and evaluating, case by case, also the costs of the overall process.

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Data Availability Enquiries about data availability should be directed to the authors.

Declarations

Competing Interests The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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