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Increase of the anaerobic biodegradability of olive mill wastewaters through a pre-treatment with hydrogen peroxide in alkaline conditions

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ABSTRACT

Olive mill wastewaters, due to a low biodegradability, acidic pH, high salinity, lack of nutrients and elevated amounts of polyphenols, are hard to treat using conventional biological processes. In recent years, several pre-treatments of these by-products have been developed to increase their anaerobic biodegradability and to exploit them for biogas production. However, these processes are often expensive and hard to carry out. This paper presents a process, easy to manage, that involves the use of hydrogen peroxide under alkaline conditions without the addition of catalysts. This process is able to efficiently increase the anaerobic biodegradability of olive mill wastewaters. In fact, with mild treatment conditions, it is possible to obtain polyphenols abatements of 78%, increases of 48% in the volatile fatty acids content and only a restricted reduction in the organic matter content. By conducting many digestion tests, it was verified that this process permits high biogas production by olive oil by-products. Indeed, with properly prepared samples, after a negligible acclimation period, methane yields of approximately 0.37 $\mathrm{LCH4/gCOD_{removed}}$ were detected.

Keywords: Anaerobic digestion; Biogas; Hydrogen peroxide; Olive mill wastewaters

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1. Introduction

In the Mediterranean region, large amounts of waste and wastewaters produced from olive milling represent an important environmental issue. Because of the high pollution load and seasonal production, these residues are not treatable by means of conventional processes [1–3]. The exploitation of olive oil wastewaters for biogas production in anaerobic digestion processes may be a suitable and profitable approach. However, the acidic pH, high salinity, lack of nutrients, low biodegradability and elevated polyphenols concentrations make it necessary to perform an adequate pre-treatment [1–4]. For the removal of phyto-toxic and low-biodegradable compounds, many advanced oxidation techniques have been proposed [5–20]. Some of these, such as ultrasonic irradiation, photo-catalysis and electrochemical processes are quite profitable because there is no need for the addition of chemical reagents. However, the solid contents typically found in olive oil wastewaters may partially impede the applicability of these processes, requiring more severe operating conditions and greater energy consumption [20].

Among the different available techniques, catalytic oxidation processes using hydrogen peroxide (Fenton and Fenton-like processes) give good results for the removal of various classes of organic pollutants and

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also in treating real concentrated wastewaters. The catalysed hydrogen peroxide decomposition results in the formation of highly reactive free radicals, particularly hydroxyl radicals, which are capable of oxidizing many organic pollutants to lower molecular weight compounds and eventually to carbon dioxide and water. Esplugas et al. [16] studied and compared the effectiveness of different advanced oxidation processes (ozone and its combination, photolysis and UV/H_2O_2, Fenton and photocatalysis) for phenol degradation and showed that the Fenton process has the fastest degradation rate. Furthermore, several research studies have been carried out to investigate the use of different transition metals in homogeneous and heterogeneous form in conjunction with hydrogen peroxide [21–29]. Many works demonstrated that the use of Fenton's processes as a primary treatment of OMW enhances the efficiency of anaerobic digestion because the pre-treated effluent is more readily degradable than the original untreated effluent. Furthermore, because of the reduction in the polyphenol content, the anaerobic processing of pre-treated OMW evolves without inhibition and achieves high methane productions [30].

However, the applicability of the Fenton process on field conditions is limited by the need to recover the metallic catalyst after the treatment. This operation, generally carried out through the chemical precipitation of metallic ions, complicates the treatment and produces a precipitate difficult to manage and expensive to dispose. The precipitate, moreover, by embedding an elevated amount of the colloidal and particulate organic matters of the wastewater, reduces the organic load available for the biogas production in the next digestion process. Also heterogeneous catalysts are difficult to recover because of the turbidity and density of olive milling residues. Furthermore, in a heterogeneous treatment, leaching of the metallic ions from the solid carrier generally occurs, making necessary further treatment for the removal of the catalyst transferred into the solution.

The present paper reports the results of an experimental investigation that defines a process able to increase the anaerobic biodegradability of OMW without the addition of catalysts. This process, unlike conventional catalytic treatments, involves the use of hydrogen peroxide under an alkaline environment. Optimizing the operating conditions, we have demonstrated that this type of treatment, which, until now, has only been applied for wastewater bleaching and the delignification of woody residues [31,32], efficiently removes the polyphenols and increases the amount of volatile fatty acids of OMW. Several tests were carried out to define more suitable pre-treatment conditions to make OMW easily degradable in anaerobic processes. The effectiveness of the developed method was verified by many digestion batch tests carried out on both pre-treated and raw olive oil wastewaters.

2. Description of the experiments

2.1. Materials and methods

The experiments were carried out on olive mill wastewaters produced by a continuous three-phase process; moreover, samples of cattle manure and cattle manure digestate were used during the experiments. The wastewaters were withdrawn from an olive mill plant and a cattle farm located near Cosenza (southern Italy) and stored in a laboratory in 25 L containers at 4°C. The characteristics of the wastewaters are summarized in Table 1. The first phase of research has been focused on the development of the pre-treatment with hydrogen peroxide (Fig. 1). After the optimal conditions for the oxidation process were identified, both the pre-treated and fresh OMW samples were subjected to many digestion tests (Fig. 1). During the experiments, caustic soda and hydrogen peroxide (analytical grade) were used.

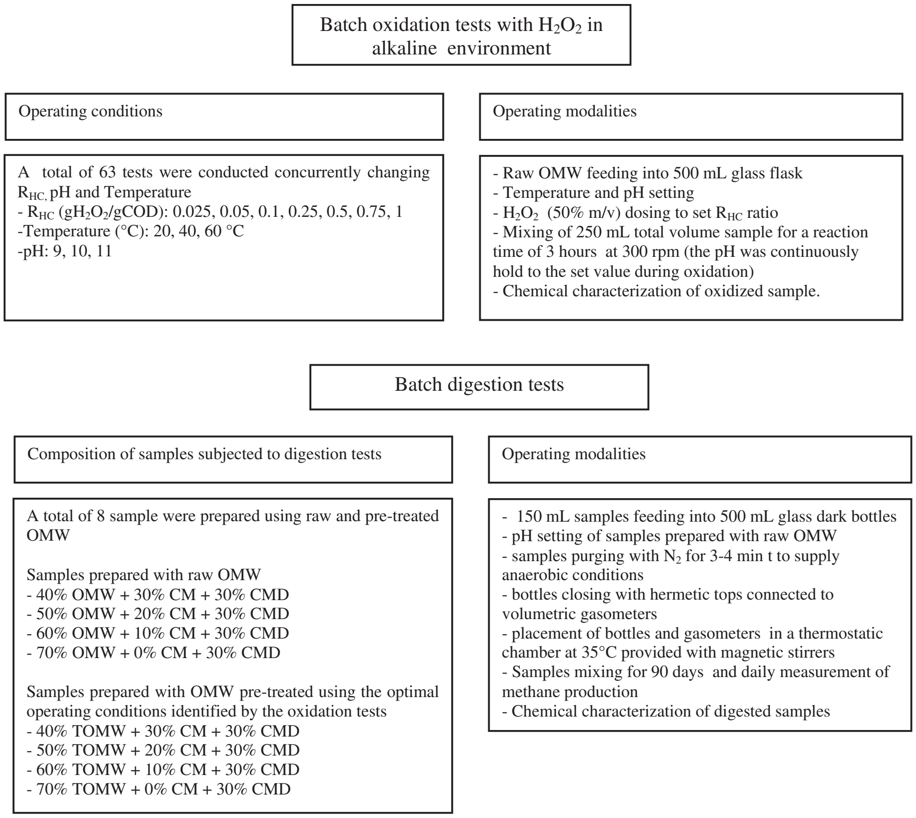
2.2. Oxidation tests

To identify the process conditions necessary to efficiently reduce the amount of polyphenols and increase the VFA content, several experiments were conducted by concurrently changing the R_{HC} value, pH and

Table 1
Characteristics of raw wastewaters used during experiments

Sample	pH	Conductivity mS/cm	COD gO_2/L	BOD_5 gO_2/L	TP $g_{gallic\ acid}/L$	VFA $g_{acetic\ acid}/L$	ALK $gCaCO_3/L$	TKN gN/L	NH_4^+-N gN/L	PO_4^{3-}-P gP/L	TS g/L	VS g/L
OMW	4.6	15.82	120.3	26.1	8.62	4.69	3.46	0.70	0.041	0.285	56.1	42.9
CM	7.4	12.86	51.8	28.3	0.57	3.17	8.41	1.69	0.60	0.291	60.9	44.5
CMD	8.3	19.01	32.5	7.8	0.74	0.67	14.63	2.13	1.99	0.337	40.7	27.3

Note: OMW: raw olive mill wastewater, CM: raw cattle manure, CMD: raw cattle manure digestate.



Batch oxidation tests with H_2O_2 in alkaline environment

Operating conditions

A total of 63 tests were conducted concurrently changing R_{HC}, pH and Temperature
- R_{HC} ($gH_2O_2/gCOD$): 0.025, 0.05, 0.1, 0.25, 0.5, 0.75, 1
- Temperature (°C): 20, 40, 60 °C
- pH: 9, 10, 11

Operating modalities

- Raw OMW feeding into 500 mL glass flask
- Temperature and pH setting
- H_2O_2 (50% m/v) dosing to set R_{HC} ratio
- Mixing of 250 mL total volume sample for a reaction time of 3 hours at 300 rpm (the pH was continuously hold to the set value during oxidation)
- Chemical characterization of oxidized sample.

Batch digestion tests

Composition of samples subjected to digestion tests

A total of 8 sample were prepared using raw and pre-treated OMW

Samples prepared with raw OMW
- 40% OMW + 30% CM + 30% CMD
- 50% OMW + 20% CM + 30% CMD
- 60% OMW + 10% CM + 30% CMD
- 70% OMW + 0% CM + 30% CMD

Samples prepared with OMW pre-treated using the optimal operating conditions identified by the oxidation tests
- 40% TOMW + 30% CM + 30% CMD
- 50% TOMW + 20% CM + 30% CMD
- 60% TOMW + 10% CM + 30% CMD
- 70% TOMW + 0% CM + 30% CMD

Operating modalities

- 150 mL samples feeding into 500 mL glass dark bottles
- pH setting of samples prepared with raw OMW
- samples purging with N_2 for 3-4 min t to supply anaerobic conditions
- bottles closing with hermetic tops connected to volumetric gasometers
- placement of bottles and gasometers in a thermostatic chamber at 35°C provided with magnetic stirrers
- Samples mixing for 90 days and daily measurement of methane production
- Chemical characterization of digested samples

Fig. 1. Experimental plan.

temperature (Fig. 1). The tests were carried out in batch mode at atmospheric pressure in a three-neck glass flask of 500 mL equipped with a mechanic stirrer. The reactor was thermostated by means of a digester, and the volatilization of the organic compounds was controlled by cooling the vapours with a water-refrigerating coil. During each test, after heating the sample and adjusting the pH with NaOH 10 N, a proper amount of 50% (w/w) hydrogen peroxide solution was added to set the R_{HC} ratio. The solution had a total volume of 250 mL and was stirred at 300 rpm for approximately 3 h to maintain the pH at the established value. A multiparameter probe housed in a glass tube was used to control the pH and temperature of the reaction mixture. The oxidized samples were analysed to determine the pollutants removal.

2.3. Digestion tests

Digestion tests were carried out to evaluate the methane productions detectable on fresh OMW and on wastewater samples pre-treated with the optimal conditions identified by means of oxidation tests. Cattle manure, as co-substrate, and cattle manure digestate, as inoculum, were used. Eight samples of 150 mL were prepared, four of which using raw OMW and four with pre-treated OMW, according to the distribution reported in Fig. 1. The experiments scheme was selected in order to evaluate the anaerobic treatability of mixtures containing growing amounts of fresh or pre-treated OMW up to completely avoid the co-substrate addition. To verify the performances of digestion process, avoiding the addition of external compounds, the tests were carried out without setting the operating parameters (COD/VS, COD:N:P and alkalinity), thus no chemical nutrients or other chemicals were provided. Only the pH of the samples prepared with raw OMW was set to approximately 7.5 using NaOH (4 N) to prevent initial acidic conditions able to inhibit the digestion process. The experiments were carried out in batch mode in 0.5 L dark glass flasks normally used for BOD tests. The bottles were equipped with hermetic closing tops connected to volumetric plexiglass gasometers by PEAD tubes. Small boxes filled with NaOH scales were placed along the connection pipes in PEAD to achieve the absorption of CO_2 and of other acid gases, so as to detect mainly the methane production during the experiments. Each bottle was charged with the reaction mixture, purged with N_2 for 3–4 min to supply anaerobic conditions and then closed and connected to gasometer. The samples were placed in a thermostatic chamber at 35℃ and continuously mixed for 90 d by magnetic stirrers. The methane volume was daily estimated by measuring the displacement of the liquid into the gasometers. After three months, the digested samples were characterized with respect to the main chemical parameters.

2.4. Analytical methods

The salinity and pH were measured by a multiparametric analyser; TS and VS by drying the sample at 105 and 550℃, respectively; the COD and alkalinity by the titrimetric methods; BOD_5 was determined by the manometric method with a respirometer (VELP FOC 225E); TKN by the Kjeldhal procedure; ammonium, nitrogen and phosphorus species by colorimetric methods using an UV spectrophotometer; VFA by the distillation method [33]; and polyphenols by the Folin and Ciocalteu procedure [34]. On the oxidized samples, the positive interference in COD measurement derived from the presence of residual H_2O_2 was estimated according to the method developed by Mantzavinos [22].

Each measurement was carried out three times, and the mean value was considered. The results of the efficiencies reported were representative of the actual removal or production of the compounds. Thus, the values were not affected by dilution because of reactant additions in the various processes.

3. Results and discussion

3.1. Oxidation pre-treatment

The results of the tests showed that, for each condition of pH and temperature, the removal of polyphenols increases with an asymptotic trend in response to the addition of H_2O_2 (Fig. 2). In particular, the abatements were between 55 and 78% for a dosage of only 0.1 $gH_2O_2/gCOD$ and then increased by approximately 10–15% with an R_{HC} ratio of approximately 0.25 (Fig. 2). Over this dosage, only a moderate growth of the efficiencies was observed (Fig. 2).

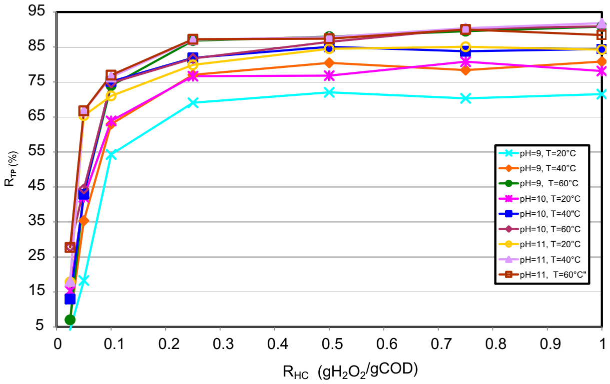


Fig. 2. Polyphenols removal (R_{TP}) detected during oxidation tests carried out changing the H_2O_2 dose (R_{HC}) at different pH and temperature values.

These results suggest that the use of high doses of peroxide is useless because it destroys the radicals produced during the treatment. Indeed, in an alkaline environment, hydrogen peroxide decomposes according to a series of reactions with the formation of $OH^{\cdot}$ (Eqs. (1–3)), which, however, can be consumed by an excess of H_2O_2 [3,7]. These considerations are in agreement with the findings of Crittenden et al. [35], who observed an attenuation of the oxidation rate in response to a high peroxide dosage in a H_2O_2/UV system.

$$H_2O_2 + OH^- \rightarrow HO_2^- + H_2O \tag{1}$$

$$H_2O_2 + HO_2^- \rightarrow OH^{\cdot} + O_2^{\cdot} + H_2O \tag{2}$$

$$H_2O_2 + OH^{\cdot} \rightarrow HO_2^{\cdot} + H_2O \tag{3}$$

The effectiveness of treatment is favoured by the pH and temperature increases, which probably enhance the production of radicals and thus the oxidation of the polyphenols. At room temperature, the pH increase accentuated the yield growth in response to the R_{HC} value (Fig. 2). In particular, changing the pH from 9 to 11, the maximum efficiencies ranged between 70 and 85% (Fig. 2). The positive effect of the pH increase on polyphenols removal was also found at a process temperature of 40℃. With these conditions, the pH increase is positive up to a value of 10, which ensures a further increase of about 5–7% in the maximum efficiency than the abatements reached at a pH of 9 (Fig. 2). Increasing the pH up to 11, a further lower augmentation in the maximum yield, around 5%, was observed. In comparison to those reached at room temperature, the abatements obtained at 40℃ were higher by approximately 8–9% at a pH of 9 and 5–6% at a pH of 10 and 11 (Fig. 2). After changing the pH at a temperature of 60℃, only limited differences were observed on the abatement curves, which had maximum values close to 90% (Fig. 2). The increase to 60℃ produced a significant growth, around 8–9%, only in the experiments carried out at a pH of 9 (Fig. 2). Thus, the influence of the temperature on the treatment performance was significant at the lower pH.

However, the high abatement between 70 and 90%, reached with a relatively low R_{HC} ratio, confirmed the effectiveness of the treatment in a basic environment. In fact, El-Gohary et al. observed abatements of phenolic compounds of approximately 84% using an H_2O_2/COD ratio equal to 1/1 in the conventional Fenton treatment [30]. The results reported by Beltrán-Heredia et al. [36] showed that by treating OMW by Fenton's peroxidation, the total phenolics content decreases up to 93% using a peroxide dosage corresponding to an R_{HC} value of 0.28 gH_2O_2/gCOD. Bettazzi et al. [11] observed maximum polyphenolic abatements of approximately 60% with H_2O_2 additions comparable to those in the present work.

In addition to the efficient polyphenols abatement, the proposed treatment led to a remarkable increase in the volatile fatty acid content. Corresponding to the polyphenols removal curves, the increasing VFA trends were positively affected by the growth of the pH and temperature and showed a rapid enhancement in response to peroxide addition up to 0.1 gH_2O_2/gCOD, and a further moderate growth with an R_{HC} value of 0.25, beyond which the influence of the dosage was negligible (Fig. 3). Maximum increases of VFA concentration of about 2.5 g/L were detected after the oxidation pre-treatment. This substantial production of VFA suggests that part of the polyphenols and other recalcitrant compounds were degraded into various easily biodegradable intermediate organic compounds without the complete mineralization occurring during the oxidation process. This conversion is an important result because of the high biodegradability of low-molecular-weight organic acids and a further biological treatment of OMW is much easier. In particular, the preliminary transformation of recalcitrant and phytotoxic compounds into a rapidly biodegradable substrate allows, during the anaerobic treatments, to reduce the length of the hydrolysis and acidification phase, enhancing consequently the methane production.

The fact that using H_2O_2 in a basic environment causes a partial conversion of recalcitrant organic matter into easily biodegradable compounds is confirmed by the moderate reduction of the COD observed in all conditions tested. Indeed, the maximum abatements detected were between 28 and 37% (Fig. 4). These yields are comparable with the results reported by other authors that applied the Fenton process in the treatment of OMW. In fact, Beltrán-Heredia et al. [36] and Bettazzi et al. [11] observed COD removals of about 20–30%, while much higher abatements were detected combining the Fenton treatment with a coagulation step [4,30,37,38].

Anyhow, the moderate reductions of organic load achievable using the proposed process is a positive aspect because, if the wastewater is subsequently subjected to the digestion process, it ensures the availability of substrate for the biogas production.

The pre-treatment with H_2O_2 causes also a reduction of solids content always lower than 25%, while an effective ammonia removal around 13% was detected during the experiments conducted at pH 10 and 11, probably due to a moderate stripping process (Table 2). After the treatment, an increase in salinity was observed, mainly attributable to the additions of base to set pH (Table 2).

Regarding the peroxide content on the oxidized samples, residual concentrations between 21 and 33% were observed using a R_{HC} ratio higher than 0.5, while negligible amounts were detected with lower dosages. The absence of residual peroxide using low dosages makes the process particularly advantageous

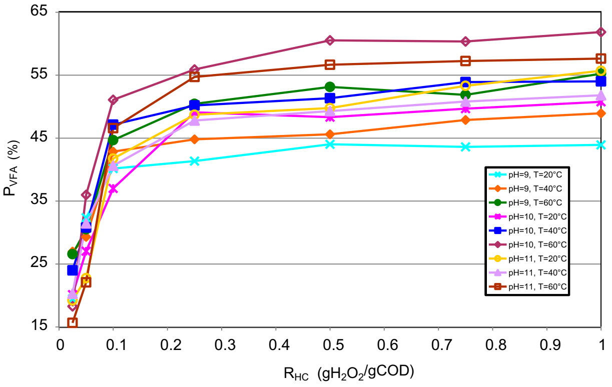


Fig. 3. VFA production (P_{VFA}) detected during oxidation tests carried out changing the H_2O_2 dose (R_{HC}) at different pH and temperature values.

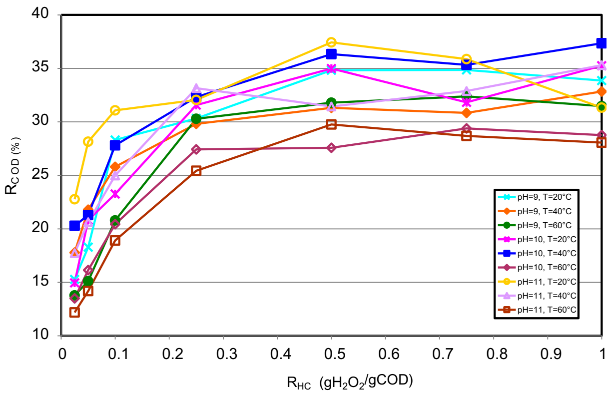


Fig. 4. COD removal (R_{COD}) detected during oxidation tests carried out changing the H_2O_2 dose (R_{HC}) at different pH and temperature values.

Table 2
Characteristics of OMW pre-treated using the optimal operating conditions identified by batch oxidation tests

Sample	pH	Conductivity mS/cm	COD gO_2/L	BOD_5 gO_2/L	TP $g_{gallic\ acid}/L$	VFA $g_{acetic\ acid}/L$	ALK $gCaCO_3/L$	TKN gN/L	NH_4^+-N gN/L	PO_4^{3-}-P gP/L	TS g/L	VS g/L
TOMW	10.06	21.92	101.1	58.3	1.896	6.96	23.25	0.64	0.034	0.227	41.5	31.33

Note: $R_{HC} = 0.1$ $gH_2O_2/gCOD$, $T = 40$°C, pH = 10.

because there is no need to take a specific step to remove it after oxidation.

Based on the results of the experiments, because a peroxide amount of 0.1 $gH_2O_2/gCOD$ gives efficiencies slightly lower than the maximum values, this dosage at a temperature of 40°C and a pH of 10 has been adopted for the treatment of the OMW to be subjected to digestion tests (Table 2). This sample was also characterized in terms of BOD_5 amount, showing a substantial enhancement of biodegradable organic matter up to 58.3 g/L (Table 2). Indeed, the BOD_5/COD ratio of raw OMW, after the pre-treatment, increased from 0.22 up to 0.58, confirming the remarkable biodegradability improvement of olive mill wastewaters.

3.2. Digestion batch tests

The main characteristics of mixtures subjected to digestion tests are reported in Table 3. The polyphenols concentrations overcome 3.8 g/L in the mixtures with raw OMW, while resulted lower than 1.5 g/L for the samples prepared with the pre-treated OMW. In these samples, the higher VFA amounts, favourable for biogas production, are also balanced by adequate alkalinity values due to the pH setting performed during oxidation process. In particular, the VFA/ALK ratios, lower than 0.25, are suitable for digestion process as stated by Khanal [39]. Anyhow, also in samples with raw OMW, after the pH adjustment to 7.5, the VFA/ALK values were compatible for anaerobic process [39]. The COD/N ratios exceeded, in some cases, the values considered necessary for anaerobic digestion [40–42], but as already stated, no nutrient compound were fed to the prepared samples. No reliable considerations can be done about the F/M (COD/VS) ratio because the VS amount of wastes used in this work is not a representative of bacterial concentrations but instead mainly of particulate organic matter.

The results of the experiments showed that there was restricted biogas production for samples prepared with raw OMW. In particular, the detected methane was negligible on samples S_3 and S_4, while a very low volume of approximately 250 mL was observed on samples S_1 and S_2 prepared with raw OMW percentages of 70 and 60%, respectively (Fig. 5).

These limited yields confirm the high inhibition power of raw olive mill wastewater. In fact, biogas production completely stopped, although the OMW was co-digested with significant amounts of cattle manure and mixed with a proper amount of inoculum. Thus, the experimental results proved that significant methane production is not achievable with an amount of raw OMW higher than 40% of the total sample volume. Indeed, also restricted COD removals were detected after the overall digestion period (Fig. 6). Consistent with these considerations, Ergüder et al. [43] demonstrated that the anaerobic biodegradation of olive mill wastewaters was effective in treating samples with COD concentrations of up to approximately 27–28 g/L. This value, assuming typical concentrations of olive mill wastewaters [44], can be obtained if a feed percentage of OMW lower than 20–25% is adopted. In experiments carried out by Hamdi [45], methane production was possible with dilution of about 5–15% (V/V), corresponding to a COD of treating samples only of 5–20 g/L.

Unlike the results obtained on raw olive mill wastewater, the pre-treated sample showed remarkable methane productions, always attaining total volumes greater than 2 L (Fig. 5). In particular, higher productions of approximately 2.6 L were detected after a period of approximately 2 months on a sample prepared with 50% treated OMW and 30% cattle manure (Fig. 5). The effectiveness of digestion was marked by the high methane yield, resulting in approximately 0.37 $LCH_4/gCOD_{removed}$ (Table 4), just lower than the theoretical value of 0.395 $LCH_4/gCOD_{removed}$ [43]. These yields are greater than those detected in other studies. In fact, on OMW pre-treated by electro-Fenton process, Khoufi et al. [14] detected, in anaerobic filters, maximum yields of 0.32–0.34 $LCH_4/gCOD_{removed}$. In our test the high efficiency was detected in spite of the high initial concentrations of about 70 gCOD/L that confirms the improvement of biodegradability of OMW after the treatment with H_2O_2 in alkaline conditions. In fact, Sabbah et al. [46] did not observe any transformation of organic matter in batch digestion

Table 3
Characteristics of the prepared samples to be subjected to digestion tests

Sample	Composition	pH	Conductivity (mS/cm)	COD (gO_2/L)	TP ($g_{gallic\ acid}$/L)	VFA ($g_{acetic\ acid}$/L)	ALK ($gCaCO_3$/L)	VFA/ALK ($g_{acetic\ acid}$/$gCaCO_3$)	TKN (gN/L)	NH_4^+-N (gN/L)	PO_4^{3-}-P (gP/L)	COD/TKN (gO_2/gN)	TS (g/L)	VS (g/L)
S_{T1}	70% TOMW + 30% CMD	8.60	21.34	81.47	1.50	5.07	21.1	0.240	1.10	0.611	0.260	74.03	40.6	29.4
S_{T2}	60% TOMW + 10% CM + 30% CMD	8.47	20.21	74.32	1.44	4.70	19.7	0.238	1.19	0.684	0.268	62.44	43.1	32.5
S_{T3}	50% TOMW + 20% CM + 30% CMD	8.53	18.92	70.60	1.30	4.32	17.6	0.245	1.30	0.746	0.273	54.28	46.8	33.8
S_{T4}	40% TOMW + 30% CM + 30% CMD	8.33	18.10	65.88	1.19	3.93	16.0	0.245	1.40	0.786	0.278	46.82	47.6	34.3
S_1	70% OMW + 30% CMD	5.62*	15.40	91.96	6.27	3.48	12.7	0.273	1.13	0.595	0.298	81.35	51.1	38.0
S_2	60% OMW + 10% CM + 30% CMD	5.85*	15.35	85.10	5.46	3.34	12.1	0.276	1.24	0.680	0.301	68.58	52.3	38.9
S_3	50% OMW + 20% CM + 30% CMD	6.30*	15.17	81.30	4.66	3.19	12.3	0.259	1.38	0.753	0.302	58.89	52.4	39.2
S_4	40% OMW + 30% CM + 30% CMD	6.63*	15.10	72.70	3.82	3.02	12.1	0.249	1.44	0.795	0.303	50.54	53.6	39.2

*The value was set to approximately 7.5 using NaOH 4 N.

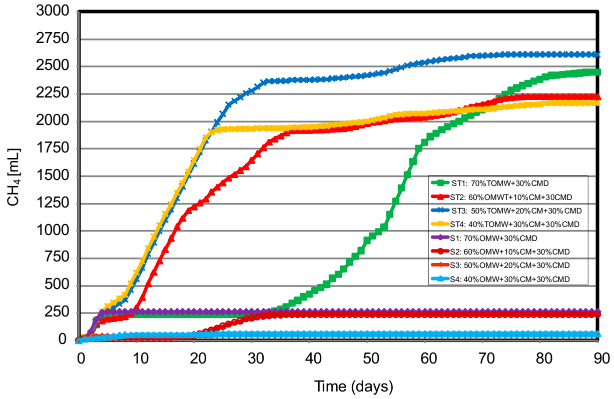


Fig. 5. Cumulative methane produced during the digestion tests.

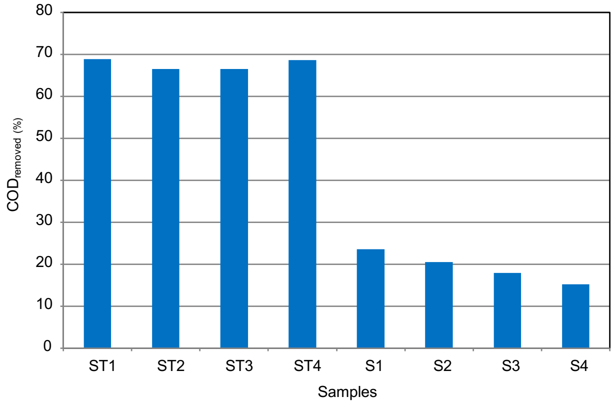


Fig. 6. COD removed after the digestion process.

tests conducted on high concentrated samples characterized by COD higher than 50 gCOD/L. Moreover in our experiments, with the aforementioned mixture, a negligible acclimation period was observed. Indeed, exponential methane production was obtained only a few days after the start of the experiment (Fig. 5). This confirms that the inhibiting power of OMW is significantly reduced by means of the adopted pre-treatment.

Similar behaviour was observed with mixture S_{T4}, composed of 40% TOMW and 30% cattle manure. However, in this case, methane production reached a cumulate volume of approximately 1.9 L after 20 d, beyond which a further moderate increase up to 2.2 L was achieved (Fig. 5). This efficiency reduction, compared to sample S_{T3}, is due to a lower initial COD, which slightly limits the overall production. Nevertheless, a methane yield of 0.32 $LCH_4/gCOD_{removed}$ was detected (Table 4).

An increase up to 60% in the amount of treated OMW reduced the production yield approximately to 0.30 $LCH_4/gCOD_{removed}$ (Table 4). The increase of OMW percentage caused also a moderate extension of the time needed for biomass acclimation. This period was considerably longer for the sample with 70% treated olive mill wastewater. Methanization started only after 20 d, beyond which, however, a rapid production phase occurred to reach a significant overall volume of 2.45 L (Fig. 5). The lower efficiency detected on samples with initial amount of OMW greater than 50% is a consequence of their worse initial properties (Table 3). In fact, these samples were characterized by conductivity over than 20 mS/cm and a COD/N ratio higher than that suggested for the anaerobic process [40,41]. These values probably influenced the performance of digestion more than the polyphenols amount that was only slightly higher than that of samples with 50% TOMW (Table 3). Anyhow, the results of digestion tests proved that the proposed pre-treatment with H_2O_2 in alkaline environment makes the next anaerobic process profitable. In fact, even though a high acclimation time, the biogas production occurred also in samples of pre-treated OMW without the addition of cattle manure. The effectiveness of digestion process was also confirmed by percentage values of COD removed, of about 67% in every mixture tested (Fig. 6). However, to maximize the methane yield and to limit the acclimation period, it is preferable to subject to digest samples with an amount of pre-treated OMW equal to half of the total volume.

Besides the high biogas production, by the digestion of pre-treated OMW, it derives a waste compatible for the utilization as fertilizer. In effect, the values of main parameters detected on digestate (Table 4) were in line with those of waste residue of digestion of cattle slurry plus some agroindustrial residues (maize-oat silage) [47]. Albuquerque et al. [47] demonstrated that the agricultural use of this type of digestate can be efficiently accomplished if appropriate application modes are considered in order to limit the salinity value.

3.3. Analysis of the chemical costs

The proposed process with the identified operating conditions makes it necessary to add 26 LH_2O_2/m^3OMW and 28 $LNaOH/m^3OMW$ for the treatment of the OMW used in this study. By analysing the Italian Market (2012 market prices), the cost of H_2O_2 (50% w/v) and NaOH (40% w/v) is 330 and 220 €/m^3, respectively [48]. Thus, the total outlay for chemical consumption is 14.74 €/m^3OMW. This cost

Table 4
Characteristics of the samples after the digestion process

Sample	Composition	pH	Cond. (mS/cm)	COD (gO_2/L)	TP ($g_{gallic\ acid}$/L)	VFA ($g_{acetic\ acid}$/L)	ALK ($gCaCO_3$/L)	TKN (gN/L)	NH_4^+-N (gN/L)	PO_4^{3-}-P (gP/L)	COD/TKN (gO_2/gN)	TS (g/L)	VS (g/L)	CH_4/COD_{rem} (L/gO_2)
S_{T1}	70% TOMW + 30% CMD	8.60	27.95	24.62	1.34	1.96	18.5	0.826	0.731	0.275	29.95	24.20	8.95	0.29
S_{T2}	60% TOMW + 10% CM + 30% CMD	8.51	26.86	24.83	1.27	1.44	17.8	0.947	0.891	0.280	13.46	26.20	10.74	0.30
S_{T3}	50% TOMW + 20% CM + 30% CMD	8.72	27.24	23.75	1.05	0.78	16.1	1.174	1.042	0.276	20.22	27.40	12.33	0.37
S_{T4}	40% TOMW + 30% CM + 30% CMD	8.70	20.25	20.75	0.97	0.77	14.0	1.285	1.056	0.368	16.14	34.60	13.84	0.32
S_1	70% OMW + 30% CMD	6.34	16.40	71.50	4.87	2.98	11.4	0.756	0.623	0.286	94.50	35.62	22.07	0.08
S_2	60% OMW + 10% CM + 30% CMD	7.77	16.12	67.94	3.98	2.69	11.1	0.878	0.732	0.297	77.35	39.20	26.66	0.09
S_3	50% OMW + 20% CM + 30% + DCM	7.76	16.50	69.56	3.45	2.65	10.9	0.972	0.847	0.306	71.54	37.00	27.04	0.02
S_4	40% OMW + 30% CM + 30% CMD	7.62	16.34	60.90	3.18	2.05	11.2	1.038	0.855	0.307	58.68	38.50	27.98	0.04

can be significantly reduced by using lime instead of caustic soda to induce the basic conditions necessary for oxidation. In fact, by preliminary experiments we estimate a lime amount approximately of 14 kg/m^3OMW to set the pH up to the optimal value for the pre-treatment of OMW. Considering the price about 85 €/ton, the cost for lime results of about 1.2 €/m^3OMW, thus, an overall outlay around to 9.77 €/m^3OMW is sufficient to carry out the pre-treatment at the optimal operating conditions.

Regarding the energy necessary to carry out the proposed oxidation process at 40°C, it could be obtained from the combustion of biogas produced by the digestion of pre-treated OMW.

A comparative analysis with other processes is hard to carry out because the expense of treatment depends on many factors such as the wastewater characteristics, the removal yields of pollutants and the operating conditions adopted.

Anyway, the proposed process has some positive aspects if compared with Fenton process. In fact for the conventional treatment, in addition to the peroxide dosage, the cost of the catalyst must be considered. Moreover, the catalyst's recovery after oxidation causes also the consumption of basic compounds to increase the pH up to values suitable for the chemical precipitation of metallic ions. In addition, the precipitate produced is difficult to manage and costly to dispose.

Results of the developed pre-treatment becomes even more attractive taking into account the economic benefit that could be obtained from the cogeneration of electric energy by means of the biogas produced during the digestion of TOMW. In fact, on the basis of the methane yield (0.37 $m^3CH_4/KgCOD_{removed}$) and the COD transformation (67%) detected during the experiments, by the digestion of TOMW of this study, a methane amount of about 25.04 m^3CH_4/m^3OMW is achievable which corresponds, assuming an electric rate of 3.52 KWh/m^3CH_4, approximately to 88.14 KWh/m^3OMW. This production determines a mean profit because in many countries an economic incentive is granted for the production of energy through digestion of biomass wastes. For example, assuming the benefit conceded by the Italian regulation (0.236 €/KWh), a proceeds of about 20.6 €/m^3OMW could be obtained. Thus, taking into account the expense for the OMW pre-treatment, a net profit, between 5 and 10 €/m^3OMW, is achievable.

4. Conclusions

The present paper defined a process with hydrogen peroxide in alkaline conditions that, without the addition of catalysts, is able to significantly improve the anaerobic treatability of olive mill wastewaters. The results of the experimental investigations demonstrated that with moderate peroxide dosages, it is possible to obtain a polyphenols removal of approximately 78%, a VFA increase of 48% and only a limited reduction in the organic load. The process is advantageous because the absence of external catalysts simplifies the treatment, and taking a specific step to remove them is unnecessary. In addition, the disposal cost associated with the formation of a bulky precipitate is prevented. Furthermore, with the operating conditions identified, the residual peroxide is negligible. Thus, there is no need for further efforts for its removal. Because of the method's effects, pre-treated OMW is particularly suitable for energetic utilization in an anaerobic process. This is confirmed by the results of the controls carried out on samples subjected to batch digestion tests, which show remarkable methane yields, up to 0.37 $LCH_4/gCOD_{removed}$, on pre-treated samples.

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Nomenclature

OMW	—	olive mill wastewater
TOMW	—	treated olive mill wastewater
CM	—	cattle manure
CMD	—	cattle manure digestate
COD	—	chemical oxygen demand (gO_2/L)
BOD_5	—	biochemical oxygen demand (gO_2/L)
ALK	—	alkalinity ($gCaCO_3/L$)
TP	—	total polyphenols ($g_{gallic\ acid}/L$)
VFA	—	volatile fatty acids ($g_{acetic\ acid}/L$)
TKN	—	total Kjeldahl nitrogen (gN/L)
TS	—	total solids (g/L)
VS	—	volatile solids (g/L)
R_{HC}	—	hydrogen peroxide/COD ratio ($gH_2O_2/gCOD$)

References

[1] M. Beccari, G. Carucci, A.M. Lanz, M. Majone, M. Petrangeli Papini, Removal of molecular weight fractions of COD and phenolic compounds in an integrated treatment of olive oil mill effluents, Biodegradation 13 (2002) 401–410.

[2] A. Fiorentino, A. Gentili, M. Isidori, M. Lavorgna, A. Parrella, F. Temussi, Olive oil mill wastewater treatment using a chemical and biological approach, J. Agric. Food. Chem. 52 (2004) 5151–5154.

[3] M. Ahmadi, F. Vahabzadeh, B. Bonakdarpour, E. Mofarrah, M. Mehranian, Application of the central composite design and response surface methodology to the advanced treatment of olive oil processing wastewater using Fenton's peroxidation, J. Hazard. Mater. 123 (2005) 187–195.

[4] B.K. Mert, T. Yonar, M.Y. Kiliç, K. Kestioğlu, Pre-treatment studies on olive oil mill effluent using physicochemical, Fenton and Fenton-like oxidations processes, J. Hazard. Mater. 174 (2010) 122–128.

[5] M. Yalılı Kılıç, T. Yonar, K. Kestioğlu, Pilot-scale treatment of olive oil mill wastewater by physicochemical and advanced oxidation processes, Environ. Technol. 34 (2013) 1521–1531.

[6] H. Iboukhoulef, A. Amrane, H. Kadi, Microwave-enhanced Fenton-like system, Cu(II)/H_2O_2, for olive mill wastewater treatment, Environ. Technol. 34 (2013) 853–860.

[7] S. Vilhunen, J. Puton, J. Virkutyte, M. Sillanpää, Efficiency of hydroxyl radical formation and phenol decomposition using UV light emitting diodes and H_2O_2, Environ. Technol. 32 (2011) 865–872.

[8] A. Siciliano, C. Ruggiero, S. De Rosa, A new integrated treatment for the reduction of organic and nitrogen loads in methanogenic landfill leachates, Process Saf. Environ. Prot. 91 (2013) 311–320.

[9] M. Drouiche, V. Le Mignot, H. Lounici, D. Belhocine, H. Grib, A. Pauss, N. Mameri, A compact process for the treatment of olive mill wastewater by combining UF and UV/H_2O_2 techniques, Desalination 169 (2004) 81–88.

[10] D. Atanassova, P. Kefalas, C. Petrakis, D. Mantzavinos, N. Kalogerakis, E. Psillakis, Sonochemical reduction of the antioxidant activity of olive mill wastewater, Environ. Int. 31 (2005) 281–287.

[11] E. Bettazzi, M. Morelli, S. Caffaz, C. Caretti, E.Azzari C. Lubello, Olive mill wastewater treatment: An experimental study, IWA 5th World Water Congress, IWA, London, UK, 2006.

[12] A. Cesaro, V. Naddeo, V. Amodio, V. Belgiorno, Enhanced biogas production from anaerobic codigestion of solid waste by sonolysis, Ultrason. Sonochem. 19 (2012) 596–600.

[13] S. Meriç, H. Selcuk, M. Gallo, V. Belgiorno, Decolourisation and detoxifying of Remazol Red dye and its mixture using Fenton's reagent, Desalination 173 (2005) 239–248.

[14] S. Khoufi, F. Aloui, S. Sayadi, Treatment of olive oil mill wastewater by combined process electro-Fenton reaction and anaerobic digestion, Water Res. 40 (2006) 2007–2016.

[15] G. Lofrano, S. Meriç, V. Belgiorno, R.M.A. Napoli, Fenton's oxidation of various-based tanning materials, Desalination 211 (2007) 10–21.

[16] S. Esplugas, J. Giménez, S. Contreras, E. Pascual, M. Rodríguez, Comparison of different advanced oxidation processes for phenol degradation, Water Res. 36 (2002) 1034–1042.

[17] I. Yahiaoui, F. Aissani-Benissad, F. Fourcade, A. Amrane, Response surface methodology for the optimization of the electrochemical degradation of phenol on Pb/PbO_2 electrode, Environ. Prog. Sustainable Energy 31 (2012) 515–523.

[18] I. Yahiaoui, F. Aissani-Benissad, K. Madi, N. Benmehdi, F. Fourcade, A. Amrane, Electrochemistry pre-treatment combined with biological treatment for the degradation of Methylene Blue dye: Pb/PbO_2 electrode and modeling-optimization through central composite design, Ind. Eng. Chem. 52 (2013) 14743–14751.

[19] I. Yahiaoui, F. Aissani-Benissad, F. Fourcade, A. Amrane, Combination of an electrochemical pre-treatment with a biological oxidation for the mineralization of nonbiodegradable organic dyes: Basic Yellow 28 dye, Environ. Prog. Sustainable Energy 33 (2014) 160–169.

[20] E. Kotta, N. Kalogerakis, D. Mantzavinos, The effect of solids on the electrochemical treatment of olive mill effluents, J. Chem. Technol. Biotechnol. 82 (2007) 504–511.

[21] L. Pecci, G. Montefoschi, D. Cavallini, Some new details of the copper-hydrogen peroxide interaction, Biochem. Biophys. Res. Commun. 235 (1997) 264–267.

[22] D. Mantzavinos, Removal of benzoic acid derivatives from aqueous effluents by the catalytic decomposition of hydrogen peroxide, Process Saf. Environ. Prot. 81 (2003) 99–106.

[23] R. Ben Achma, A. Ghorbel, A. Dafinov, F. Medina, Copper-supported pillared clay catalysts for the wet hydrogen peroxide catalytic oxidation of model pollutant tyrosol, Appl. Catal., A 349 (2008) 20–28.

[24] I.A. Salem, Kinetics of the oxidative color removal and degradation of bromophenol blue with hydrogen peroxide catalyzed by copper(II)-supported alumina and zirconia, Appl. Catal., B 28 (2000) 153–162.

[25] I.U. Castro, F. Stüber, A. Fabregat, J. Font, A. Fortuny, C. Bengoa, Supported Cu(II) polymer catalysts for aqueous phenol oxidation, J. Hazard. Mater. 163 (2009) 809–815.

[26] R.M. Liou, S.H. Chen, CuO impregnated activated carbon for catalytic wet peroxide oxidation of phenol, J. Hazard. Mater. 172 (2009) 498–506.

[27] S. De Rosa, G. Giordano, T. Granato, A. Katovic, A. Siciliano, F. Tripicchio, Chemical pretreatment of olive oil mill wastewater using a metal-organic framework catalyst, J. Agric. Food Chem. 53 (2005) 8306–8309.

[28] S. De Rosa, A. Siciliano, A catalytic oxidation process of olive oil mill wastewaters using hydrogen peroxide and copper, Desalin. Water Treat. 23 (2010) 187–193.

[29] G. Giordano, S. Perathoner, G. Centi, S. De Rosa, T. Granato, A. Katovic, A. Siciliano, A. Tagarelli, F. Tripicchio, Wet hydrogen peroxide catalytic oxidation of olive oil mill wastewaters using Cu-zeolite and Cu-pillared clay catalysts, Catal. Today 124 (2007) 240–246.

[30] F.A. El-Gohary, M.I. Badawy, M.A. El-Khateeb, A.S. El-Kalliny, Integrated treatment of olive mill wastewater (OMW) by the combination of Fenton's reaction and anaerobic treatment, J. Hazard. Mater. 162 (2009) 1536–1541.

[31] R.C. Sun, J.M. Fang, J. Tomkinson, Delignification of rye straw using hydrogen peroxide, Ind. Crops Prod. 12 (2000) 71–83.

[32] S.C. Rabelo, N.A. Amezquita Fonseca, R.R. Andrade, R. Maciel Filho, A.C. Costa, Ethanol production from enzymatic hydrolysis of sugarcane bagasse pretreated with lime and alkaline hydrogen peroxide, Biomass Bioenergy 35 (2011) 2600–2607.

[33] APHA, Standard Methods for the Examination of Water and Wastewater, 20th ed., Washington, DC, 1998.
[34] O. Folin, V. Ciocalteu, On tyrosine and tryptofan determination in protein, J. Biol. Chem. 73 (1927) 627–650.
[35] J.C. Crittenden, S. Hu, D.W. Hand, S.A. Green, A kinetic model for H_2O_2/UV process in a completely mixed batch reactor, Water Res. 33 (1999) 2315–2328.
[36] J. Beltrán-Heredia, J. Torregrosa, J. García, J.R. Domínguez, J.C. Tierno, Degradation of olive mill wastewater by the combination of Fenton's reagent and ozonation processes with an aerobic biological treatment, Water Sci. Technol. 44(5) (2001) 103–108.
[37] L. Rizzo, G. Lofrano, M. Grassi, V. Belgiorno, Pretreatment of olive mill wastewater by chitosan coagulation and advanced oxidation processes, Sep. Purif. Technol. 63 (2008) 648–653.
[38] W.K. Lafi, M. Al-Anber, Z.A. Al-Anber, M. Al-Shannag, A. Khalil, Coagulation and advanced oxidation processes in the treatment of olive mill wastewater (OMW), Desalin. Water Treat. 24 (2010) 251–256.
[39] S. Khanal, Anaerobic Biotechnology for Bioenergy Production: Principles and Applications, Wiley-Blackwell, Oxford, 2008.
[40] M. Henze, P. Harremoes, Anaerobic treatment of wastewater in fixed film reactors—A literature review, Water Sci. Technol. 15(8–9) (1983) 1–101.
[41] M.A. Dareioti, S.N. Dokianakis, K. Stamatelatou, C. Zafiri, M. Kornaros, Biogas production from anaerobic co-digestion of agroindustrial wastewaters under mesophilic conditions in a two-stage process, Desalination 248 (2009) 891–906.
[42] R.E. Speece, Anaerobic Biotechnology for Industrial Wastewaters, Archae Press, Nashville, TN, 1996.
[43] T.H. Ergüder, E. Güven, G.N. Demirer, Anaerobic treatment of olive mill wastes in batch reactors, Process Biochem. 36 (2000) 243–248.
[44] J.A. Morillo, B. Antizar-Ladislao, M. Monteoliva-Sánchez, A. Ramos-Cormenzana, N.J. Russell, Bioremediation and biovalorisation of olive-mill wastes, Appl. Microbiol. Biotechnol. 82 (2009) 25–39.
[45] M. Hamdi, Toxicity and biodegradability of olive mill wastewaters in batch anaerobic digestion, Appl. Biochem. Biotechnol. 37 (1992) 155–163.
[46] I. Sabbah, T. Marsook, S. Basheer, The effect of pretreatment on anaerobic activity of olive mill wastewater using batch and continuous systems, Process Biochem. 39 (2004) 1947–1951.
[47] J.A. Albuquerque, C. de la Fuente, A. Ferrer-Costa, L. Carrasco, J. Cegarra, M. Abad, M.P. Bernal, Assessment of the fertiliser potential of digestates from farm and agroindustrial residues, Biomass Bioenergy 40 (2012) 181–189.
[48] A. Siciliano, S. De Rosa, Recovery of ammonia in digestates of calf manure through a struvite precipitation process using unconventional reagents, Environ. Technol. 35 (2014) 841–850.