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Improving the Anaerobic Digestion of Wine-Industry Liquid Wastes: Treatment by Electro-Oxidation and Use of Biochar as an Additive

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Received: 13 October 2020; Accepted: 13 November 2020; Published: 16 November 2020

Abstract: Wine lees have a great potential to obtain clean energy in the form of biogas through anaerobic digestion due to their high organic load. However, wine lees are a complex substrate and may likely give rise to instabilities leading to failure of the biological process. This work analysed the digestion of wine lees using two different approaches. First, electro-oxidation was applied as pre-treatment using boron-doped diamond-based electrodes. The voltage was 25 V and different treatment times were tested (ranging from 0.08 to 1.5 h) at 25 °C. Anaerobic digestion of wine lees was evaluated in batch tests to investigate the effect of electro-oxidation on biogas yield. Electro-oxidation exhibited a significant positive effect on biogas production increasing its value up to 330 L kg^{-1} of volatile solids after 1.5 h of treatment, compared to 180 L kg^{-1} of volatile solids measured from raw wine lees. As a second approach, the addition of biochar to the anaerobic digestion of wine lees was investigated; in the experimental conditions considered in the present study, the addition of biochar did not show any positive effect on anaerobic digestion performance.

Keywords: anaerobic digestion; wine lees; pre-treatment; biochar; biogas

1. Introduction

Nowadays, Italy, France and Spain are the three top wine producers worldwide, with 14.8 million m^3 of wine produced in 2018 [1]. The winemaking industry generates large quantities of solid waste and wastewater. Solid residues derived from this industry include stalks from destemming, grape marcs (or pomace) from pressing, and lees from the settling of leftover products from the fermentation and dead yeast cells on the bottom of the vessel. The transformation of 1000 kg of processed grape produces 0.75 m^3 of wine and also 130 kg of marc, 60 kg of lees, 30 kg of stalks, and 1.65 m^3 of wastewater [2]. Winery wastes may be used to extract valuable chemicals (e.g., phenols, antioxidants, tartaric acid, lignocellulose), as feedstock for the production of bioenergy by means of thermo-chemical and biological processes, and for agricultural and environmental applications (composting, animal feed, biosorbents) [3–5]. In Europe, wine wastes as pomace and lees were commonly used in distilleries according to EC 1493/1999. Nowadays, European Council Regulations 479/2008/EC and 555/2008/EC have permitted many of the aforementioned applications.

Anaerobic digestion (AD) is an established technology able to convert biowaste into biogas, a renewable energy source, and digestate, a potential soil improver. However, AD in many circumstances

is unable to be cost-competitive and still presents several challenges that need to be overcome [6]; such as limitations due to the hydrolysis of complex compounds, the presence of inhibitory substances and the accumulation of recalcitrant components in the fermentation medium [7]. Other problems also include proper management of digestate and high costs associated with biogas cleaning and upgrading. Different strategies have been reported in the literature to counteract these drawbacks, such as the optimization of working parameters, co-digestion, and the application of pre-treatments or additives. AD has been widely studied for the treatment of winery wastewater [8] and wine solid residues as seen in Table 1. Batch digestion tests under mesophilic conditions (35–40 °C) have been traditionally evaluated reporting a wide variety of methane yields, ranging from 0.1 to 0.5 m^3 kg^{-1} of volatile solids (VS). Most studies concern the digestion of grape pomace, and few refer to wine lees (WL). Jasko et al. [9] reported specific biogas production (SBP) values ranging from 0.254 to 0.856 m^3 kg^{-1} VS (55–60% methane) when co-digesting WL at a percentage of 10–20%. Da Ros et al. [10] observed a specific methane production (SMP) of 0.370 m^3 CH_4 kg^{-1} VS from WL in AD at 55 °C.

WL, consisting of dead yeast cells, tartrates, proteins, polysaccharides, and other substances produced and settled during the fermentation [18], are without doubt a complex substrate for AD and may likely create process instability. The high COD and low pH values, associated with the high concentration of organic acids and polyphenols, may seriously affect methane production. For this reason, some studies investigated the co-digestion of WL with waste-activated sludge [11] or their co-digestion with wine wastewater sludge [12] and other substrates [19]. Although AD has been proved to be an effective treatment option for the valorisation of winery wastes, to the authors' knowledge only few studies have focused specifically on WL (see Table 1), therefore they can be regarded as an undervalued residue. There is still a need to find an efficient method to counteract the toxicity of some of the recalcitrant compounds present in WL to achieve complete valorisation of this waste.

Electrochemical oxidation, also known as electro-oxidation (EO), treatments experienced an increasing interest in the research community because this technology is able to reduce the content of refractory substances present in biowastes. EO is an attractive technology due to its ability to treat (under moderate conditions, ambient temperature and pressure) toxic and/or complex organic pollutants present in industrial or domestic wastewaters. A crucial advantage of EO is attaining the oxidation of organic substances without the need for adding chemicals, leaving therefore no toxic residues in the effluent stream.

Electro-oxidation of organic substances occurs in association with the transfer of oxygen from water to the reaction products. Water is the source of oxygen atoms for complete oxidation. The electrode produces hydroxyl radicals (Equation (1) and Figure 1), which are the intermediaries for the oxygen evolution reaction (Equation (2)) and are also responsible for the oxidation of the organic matter by means of the interaction between organic pollutants and hydroxyl radicals (Equation (3)). These interactions are strongly linked with the anode surface [20,21].

$$H_2O + \text{Electrode} \rightarrow \text{Electrode}(\cdot OH) + H^+ + e^- \quad (1)$$

$$\text{Electrode}(\cdot OH) \rightarrow \text{Electrode} + \frac{1}{2}O_2 + H^+ + e^- \quad (2)$$

$$\text{Organic compounds}_{aq} + \text{Electrode}(\cdot OH)_{\frac{a}{2}} \rightarrow \text{Electrode} + \text{Oxidised products} + \frac{a}{2}H^+ + e^- \quad (3)$$

Table 1. Overview of literature studies exploring anaerobic digestion (AD) of wine-making industry wastes. Reporting the volume of the reactor, hydraulic retention time (HRT), organic loading rate (OLR), conditions of the digestion and gas production reported by the authors.

Substrate	Feeding Mode	Experimental Conditions	Temperature (°C)	Inoculum	Substrate/Inoculum (S/I)	Methane—Biogas Production	Reference
Wine lees	batch	-	55		-	0.370 m^3CH_4 kg^{-1} VS	[10]
Grape pomace	batch	-	55		-	0.340 m^3CH_4 kg^{-1} VS	
Grape stalk	batch	-	55		-	0.130 m^3CH_4 kg^{-1} VS	
Wine lees	batch	volume: 0.7 L	37	mesophilic digestate	10%, 15%, 20% of wine lees	CH_4 content: 55–60% Biogas prod.: 0.254–0.856 m^3 kg^{-1} VS	[9]
Wine lees (75% OLR) + Waste activated sludge	cont.	volume: 230 L HRT: 21 d OLR: 2.8 kg COD m^{-3} d^{-1}	37	-	-	CH_4 content: 65% Biogas prod.: 0.38 m^3 kg^{-1} COD_{fed}	[11]
Wine lees (80% OLR) + Wine wastewater sludge (20% OLR)	cont.	working volume: 230 L OLR: 3.2 kg COD m^{-3} d^{-1} HRT: 23 d	37	mesophilic digestate	-	Biogas prod.: 0.386 m^3 kg COD Biogas prod. Rate: 1.2 m^3 m^{-3}d	[12]
Wine lees (40%) + Pomace (60%)	fed batch	working volume: 3 l 3% TS content	35	mesophilic digestate	-	CH_4 content: 62 ± 1.61% Biogas prod.: 0.89 ± 0.02 Nm^3 kg^{-1} VS	[13]
Grape pomace	batch	volume: 0.5 L	37	digestate	1:3 (COD)	CH_4 prod: 0.205 m^3 CH_4 kg^{-1} VS	[14]
Grape pomace (9 varieties)	batch	volume: 0.5 L	37	digestate	1:3 (COD)	CH_4 prod: 0.104–0.242 m^3 CH_4 kg^{-1} COD	[15]
Grape pomace	batch	volume: 0.5 L	37	digestate	1:3 (COD)	CH_4 prod: 0.131 m^3 CH_4 kg^{-1} COD	[16]
Grape pomace (2 varieties)	batch	volume: 0.5 L VS: 16.67 g L^{-1} substrate + inoculum + medium sol.	35–37	digestate	1:2 (VS)	Biogas prod.: 0.322–0.406 m^3 kg^{-1} VS CH_4 content: 49–67%	[17]

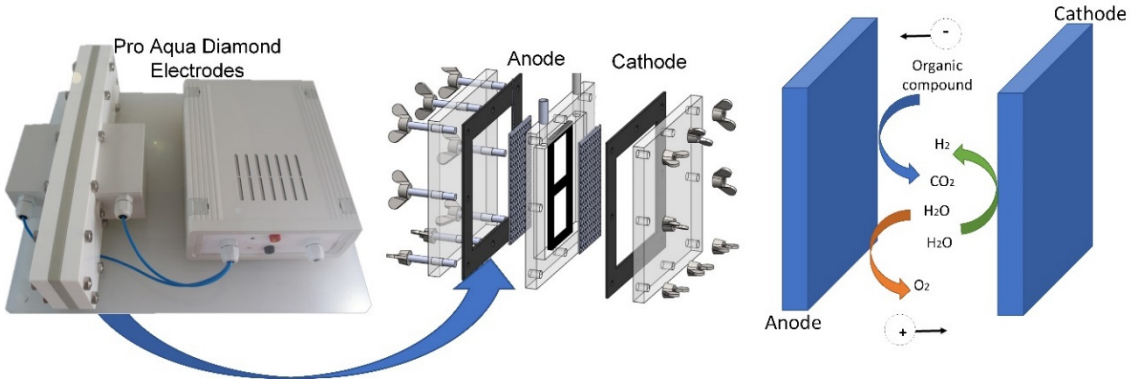

Figure 1. Pro Aqua diamond electrodes and electro-oxidation chamber scheme.

Boron-doped diamond (BDD) electrodes have shown interesting properties due to their capability to non-specifically oxidize AD inhibitors [21]. BDD electrodes oxidize the organic matter through the hydroxyl radical (OH•), a powerful oxidizing agent. BDD electrodes have good chemical stability, a wide potential window and high electrical conductivity, making them suitable to work with high organic loads and complex substrates [22].

BDD electrodes are commonly used for electrochemical wastewater treatment because of their efficient ability in total organic carbon (TOC) removal [21]. Recent studies did not show clear degradation pathways for macromolecules; generally, attention has been paid to organic matter reduction (total organic carbon, chemical oxygen demand) or specific changes in the concentration of particular compounds, as polyphenols, alcohols or melanoidins [23–25]. Nevertheless, different studies are mainly focused into the pathway of degradation of a single molecule; Cañizares et al., 2005 [26] investigated the electrochemical oxidation of phenol, found that oxalic acid was the main intermediate in the oxidation of aromatic compounds, and suggested that the aromatic ring cleavage is faster than the oxidation of carboxylic acids. The studies of Nasr et al., 2005 [27] with hydroquinone resulted in a non-detection of aromatic intermediates, even with small quantities of charge applied.

EO has been studied as pre-treatment of AD with the aim of accelerating the rate-limiting step of hydrolysis when recalcitrant substrates are to be treated [28] or reducing the toxicity of some organic compounds that may be present in winery waste.

On the other hand, biochar (BC) is the solid residue derived from the thermo-chemical processing of biomass in the absence or with limited air. Recently, the suitability of using BC as an additive in AD has attracted growing attention in the scientific community [29–31]. Among the different additives available, BC is cost-effective and in recent years the use of carbon conductive materials in AD processes was proven as an interesting way for improving performance without greatly affecting the energy demand of the process [32]. To date, many studies verified the role of BC in increasing methane production from different substrates, suggesting different mechanisms of interaction with anaerobic microflora: serving as support media for the immobilisation of biomass, promoting syntrophic metabolisms between microbial populations, increasing the buffer capacity of the digestion system and mitigation of potential inhibitors [33,34].

The main objective of this study was to investigate the improvement of AD of WL by two different approaches: one was the application of EO as pre-treatment and the other was the use of BC as an additive. EO performance was assessed through the measurement of colour removal and decrease in organic substances' concentration. Despite anaerobic digestion of WL already being studied, the novelty of this work concerns two main issues: (1) the comparison of coupled processes (AD-EO and AD-BC) as alternatives and (2) mild pre-treatment process conditions, as low treatment time (from 0.08 to 1.5 h) and low current density (from 11 to 18 mA cm^{-2}), in order to observe better, efficient, and more economic conditions for WL treatment compared to AD alone. AD performance was evaluated by means of specific methane production. In detail, the present study aimed to address

the following research questions (RQ): (RQ1) Is AD appropriate for the valorisation of WL from a technical perspective, considering potential inhibitors? (RQ2) Could EO through BDD anodes act as an effective pre-treatment to improve the performance of AD of WL? (RQ3) Can BC be a valuable additive to improve the performance of AD of WL? (RQ4) Is the effect of BC associated with its physical properties rather than with the electro-chemical ones?

2. Materials and Methods

2.1. Inoculum and Substrate Characterisation

Anaerobic digestion tests were performed at the University of Leon, Spain and at the Politecnico di Torino, Italy (Table 2). The inoculum was obtained from mesophilic digesters at wastewater treatment plants (WWTPs) in León (Spain) and Biella (Italy). Inoculum from Spain had a content of 21.1 ± 0.05 g L^{-1} total solids (TS) and 67.3% of volatile solid (VS) (expressed as % TS). The inoculum used in Italy had a solid content of 31.1 g L^{-1} TS and 43.1% VS (expressed as % TS). Wine Lees (WL) were sampled in July 2019 from a winemaking company located in the Langhe region (Piedmont, Italy) dedicated to *Barbaresco* wine production.

Table 2. Characterisation of inoculums and substrate used in batch digestion tests.

Parameter (Mean ± StD)	Inoculum (Spain)	Inoculum (Italy)	Wine Lees WL
pH	7.22 ± 0.02	7.09 ± 0.02	3.60 ± 0.02
Conductivity (mS cm^{-1})	3.55 ± 0.03	15.41 ± 0.03	2.76 ± 0.03
NH^{+4}-N (mg L^{-1})	773 ± 39	200 ± 10	25.94 ± 0.89
Total organic carbon (mg L^{-1})	288 ± 14	n.a.	67135 ± 3356
Chemical oxygen demand (g L^{-1})	24.28 ± 1.65	49.21 ± 2.46	271 ± 14
Nitrates (g L^{-1})	21.34 ± 1.07	n.a.	3.22 ± 0.16
Organic matter (%)	n.a.	n.a.	8.87 ± 0.44
Kjeldahl nitrogen (%)	n.a.	n.a.	0.09 ± 0.01

n.a.: not analysed.

2.2. Electro-Oxidation (EO) Pre-Treatment

EO of WL was carried out under batch conditions using a 70 mL cell containing two boron-doped diamond (BDD) electrodes (Pro Aqua Diamond Electrodes, Niklasdorf, Austria) used as anode and cathode. The BDD electrodes had 42 cm^{-2} effective surfaces and were placed at a 5 mm distance (Figure 1). The temperature of pre-treatments was 25 ± 1 °C, and a voltage of 25 V was applied for durations between 0.08 and 1.5 h (equivalent to current densities from 11 to 18 mA cm^{-2}). The experimental conditions of EO tests were chosen according to previous experiments (data not shown). The current efficiency (C_E), expressed as a percentage (%), was calculated from the measured total organic carbon (TOC) values and using the equation proposed by Bensalah et al. [35]:

$$C_E = (TOC_t - TOC_{t+\Delta t})/8I\Delta t \times FV \times 100 \quad (4)$$

where TOC_t and $TOC_{t+\Delta t}$ are the experimental values measured for the wine lees at times t and t + Δt (mg L^{-1}), Δt is the electrooxidation time (s), I is the current (A), F is the Faraday constant (96,485 c mol^{-1}) and V is the volume of the solution (L).

Electro-oxidation experiments were labelled according to the sample (wine lees (WL) for control sample) and for treated samples, adding the time of electrooxidation applied. The treatment time was in the range from 0.08 to 1.5 h (5 to 90 min). Therefore, assay denoted as WL_0.08 h, refers to wine lees treated for 0.08 h. Anaerobic digestion test digesters were labelled according to this same nomenclature.

2.3. Biochar and Pumice Stone as Additives

Biochar (BC) derived from softwood pellets (SWP550) obtained in a pilot-scale rotary kiln pyrolysis unit at 550 °C in the UK Biochar Research Centre (Edinburgh, UK) was employed in this study (Table 3). Aside from physical properties, BC has also chemical properties. Therefore, aiming at investigating the net chemical contribution of BC, a chemically inert material (granular pumice stone, PS, purchased from Bonsai Shopping) was also used in the AD tests. Both materials were manually powdered in an agate mortar and added separately to the digesters in a concentration of 10 g L^{-1} as described by Martínez et al. [29] and Gómez et al. [36]. Powdered pumice stone had total surface area equal to 6.29 m^2 g^{-1}.

Table 3. Characterisation of biochar SWP550.

Parameter	Mean ± StD	Parameter	Mean ± StD
Moisture (% wt)	1.52 ± 0.16	H:C_{tot} (molar ratio)	0.39 ± 0.01
Total ash (% wt)	1.25 ± 0.42	O:C_{tot} (molar ratio)	0.09 ± 0.01
Volatile Matter (% wt)	14.20 ± 0.81	Electric conductivity (dS m^{-1})	0.09 ± 0.03
Total Surface Area (m^2 g^{-1})	26.40	pH (-)	7.91 ± 0.30
C_{tot} (% wt)	85.52 ± 1.22	Total N (% wt)	<0.10
H (% wt)	2.77 ± 0.09	Total P (% wt)	0.06 ± 0.04
O (% wt)	10.36 ± 1.19	Total K (% wt)	0.25 ± 0.07

Digestion reactors were labelled as follows: WL for raw substrate, Bio_WL for wine lees supplemented with 10 g L^{-1} of biochar and PS_WL for wine lees supplemented with 10 g L^{-1} of pumice stone.

2.4. Anaerobic Digestion (AD) Tests

AD tests were carried out in Spain (August 2019) and in Italy (November 2019), adopting batch mode at 37 °C and Duran glass bottles or Erlenmeyer flasks (250 mL working volume) connected to 2 L inert-foil gas-bags (Supelco, Bellefonte PA, USA). Each reactor was filled with inoculum and substrate at a 1:1 ratio (expressed as volatile solids, VS) and 10 g L^{-1} BC. $NaHCO_3$ was added to adjust pH at 7.5. The temperature was controlled by a water bath set at 37 ± 1 °C and mixing was provided by means of magnetic stirring (RO15, IKA, Staufen, Germany). For every set of reactor, three bottles were destined for biogas measurement by water displacement with a Drechsel bottle containing a salt saturated, 5% sulfuric acid solution with methyl orange [37,38], and three bottles were destined for methane measurement. Biogas and methane monitoring happened differently in Spain and in Italy. In Spain, biogas was characterized through gas chromatography (GC-FID) (see Section 2.5); in Italy, each reactor for methane measurement was connected to a 100 mL glass bottle containing an alkaline washing solution (3N NaOH) and the outgoing gas flow was measured by water displacement. In all tests, biogas and methane production from the inoculum were evaluated along the tests. Biogas and methane volumes were measured every 2–7 days and corrected to standard temperature and pressure (STP, 0 °C and 100 kPa). Net values of biogas and methane production were calculated subtracting the contributes of the inoculum.

The performance and kinetics of anaerobic digestion in batch mode can be examined by many models, based on the assumption that bacterial growth is proportional to the production of biogas. In this study, the cumulative biogas production curves were fitted by the modified Gompertz model according to the equation:

$$B(t) = P \exp\{-\exp[(R_{max}\, e)/P\, (\lambda - t) + 1]\} \quad (5)$$

where B(t) is the cumulative biogas production (Nm^3 $kgVS^{-1}$) at time t (day); P is the biogas potential of the substrate (Nm^3 kg^{-1} VS); R_{max} is the maximum biogas production rate (Nm^3 kg^{-1} VS d^{-1}); λ is the lag phase (day); e is the base of the natural logarithm.

The goodness of fit of the functions was estimated by two parameters, the coefficient of determination (R^2) and the standard error of the estimates (SEE) [39], defined respectively in Equations (6) and (7):

$$R^2 = 1 - \sum_{i=1}^{n}\left(Y_p - \overline{Y}\right)^2 / \sum_{i=1}^{n}\left(Y_o - \overline{Y}\right)^2 \quad (6)$$

$$SEE = \sqrt{\sum_{i=1}^{n}\left(Y_o - Y_p\right)^2 / n - m} \quad (7)$$

where Y_p and Y_o are the predicted and experimental data, respectively; $\overline{Y}$ is the arithmetic mean of the experimental data; n and m are respectively the numbers of experimental values and parameters. The SEE is the standard deviation of the residual values, i.e., the difference between the experimental and predicted values.

2.5. Analytical Techniques and Procedures

Total solids (TS), volatile solids (VS), pH, conductivity, nitrogen, ammonium and organic matter contents were measured according to the American Public Health Association Standard Methods [40]. In this study, volatile solid values were used to estimate the cumulative biogas potential. Different authors reported that the method of oven drying gives inaccurate values of volatile solids when the sample contains high values of volatile fatty acids [41]. Deviations in VS values caused by the effect of volatilisation of fatty acids when performing these measurements were corrected by adding the amount of total volatile fatty acid concentration to the value measured of volatile solids by the oven dry method, thus methane potential considered VS obtained from oven dry measurements and those associated with the presence of volatile fatty acids (VFAs) in the sample.

Total organic carbon (TOC) and inorganic carbon (IC) were analysed by a high-performance analyser multi N/C®. (Analytik Jena, Thuringia, Germany), Chemical Oxygen Demand (COD) was measured with Hach Lange tubes LCK 514 (1000–10,000 mg L^{-1}) (HACH LANGE GmbH, Düsseldorf, Germany) by a high-performance spectrophotometer DR 3900 (Hach Lange, Barcelona, Spain). VFAs were analysed through a GC-FID Agilent Technologies 7890B gas chromatograph equipped with a Nukol capillary column (30 m × 0.25 mm × 0.25 mm) from Supelco as described in a previous study [42]. Ethanol was measured by an Agilent Technologies 7890B GC-FID gas chromatograph equipped by an Agilent CP97713 column (25 m × 0.25 mm × 0.2 µm) (Agilent Technologies, Santa Clara, CA, USA) Colour removal was assessed through changes in the absorbance in the range of 400–800 nm on a Beckman DU640 spectrophotometer (Beckman-Coulter, Brea, CA, USA) based on the colour associated with melanoidins which is recorded at 475 nm [43,44]. Total polyphenols (TP) were measured by colourimetry at 760 nm on a Beckman DU640 spectrophotometer. Biogas was measured by a Varian CP-3800 GC-TCD gas chromatograph (Varian, Crawley, UK) equipped with a HayeSep Q 80/100 4 m length column and molecular sieve (1.0 m × 1/8″ × 2.0 m) column (Restek Coporation, Bellefonte, PA, USA).

2.6. Sensitivity Analysis

All analyses were carried out in triplicates and average values were reported along with standard deviations. Statistical tests of experimental data were carried out using data analysis extension of Microsoft Excel 2016. The kinetic parameters were estimated using non-linear regression analysis by means of the SOLVER function of Microsoft Excel 2016. This function fits experimental data with the method of least-squares.

3. Results and Discussion

3.1. Performance of EO Pre-Treatments

The results of EO pre-treatment tests (Table 4 and Figure 2a–d) showed a rapid decrease in the content of the different parameters as the EO duration increased, with the exception of the IC parameter (which was unaltered) and VFAs, particularly acetic acid concentration, which increased. For all cases studied, TOC and COD decreased progressively under prolonged EO time achieving 20% organic material removal (Figure 2a). Nevertheless, these results were lower compared with other studies where EO was evaluated as a pre-treatment in the degradation of WL [25,45].

Table 4. Results of electro-oxidation (EO) tests.

Parameters	WL	WL_0.08 h	WL_0.5 h	WL_1.0 h	WL_1.5 h
pH	3.68 ± 0.02	3.76 ± 0.02	3.57 ± 0.02	3.33 ± 0.02	3.43 ± 0.02
Conductivity (mS cm^{-1})	2.34 ± 0.03	2.53 ± 0.03	2.61 ± 0.03	2.06 ± 0.03	2.08 ± 0.03
Total organic carbon (g L^{-1})	68.11 ± 2.89	62.57 ± 3.13	59.94 ± 2.99	52.49 ± 2.62	50.79 ± 2.83
Inorganic carbon (mg L^{-1})	700 ± 21	917 ± 45	903 ± 46	929 ± 45	910 ± 47
Chemical oxygen demand (g L^{-1})	284 ± 9	268 ± 10	263 ± 12	252 ± 7	212 ± 8
Volatile Fatty Acids (propionic and butyric acids) (mg L^{-1})	1982 ± 99	2129 ± 106	2456 ± 122	2535 ± 126	3221 ± 161
Acetic acid (mg L^{-1})	1090 ± 55	1172 ± 57	1357 ± 68	1395 ± 70	1772 ± 89
Color at 475 nm	0.79 ± 0.04	0.65 ± 0.03	0.63 ± 0.01	0.61 ± 0.03	0.60 ± 0.04
Ethanol (g L^{-1})	39.22 ± 1.96	39.93 ± 1.99	33.35 ± 1.67	24.06 ± 1.22	27.11 ± 1.35
Polyphenols (mg of Gallic acid equivalents GAE L^{-1})	58.02 ± 2.90	39.31 ± 3.11	27.69 ± 2.84	23.03 ± 3.51	20.15 ± 3.04

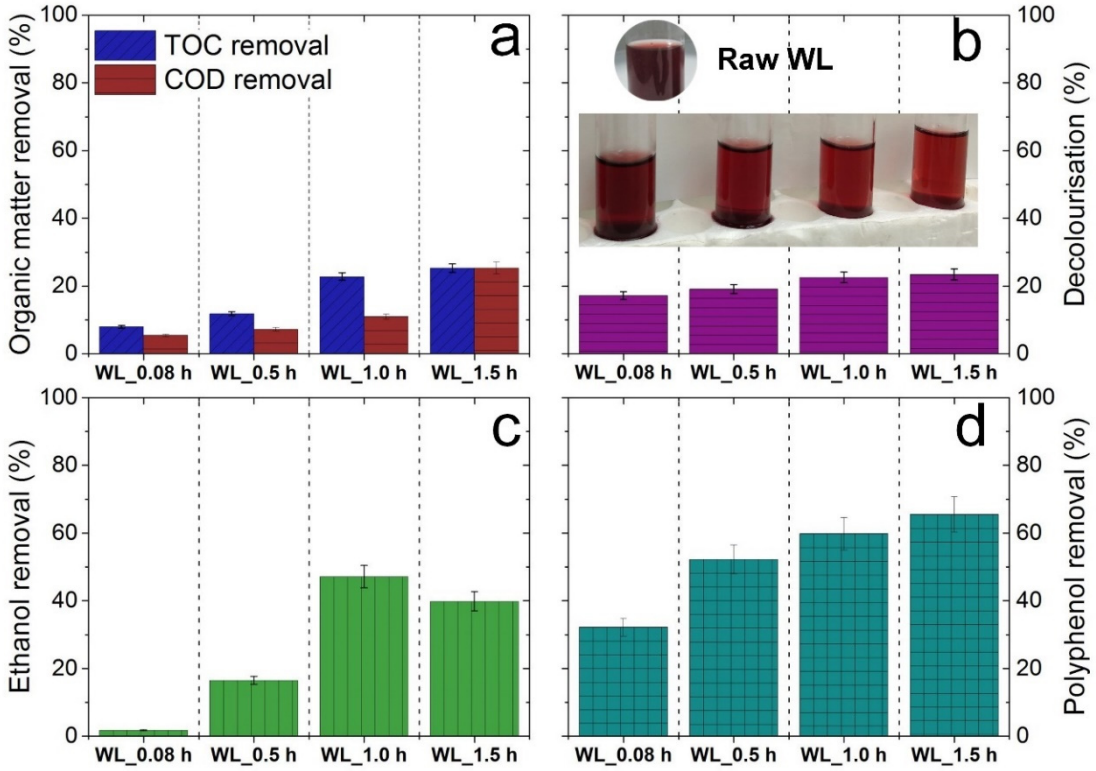

Figure 2. Removal of organic substances and colour after electro-oxidation pre-treatment tests: (**a**) Chemical oxygen demand (COD) and total organic carbon (TOC); (**b**) decolourisation at 475 nm; (**c**) ethanol; (**d**) total polyphenols.

The characteristic dark colour of WL (Figure 2b) is due to the presence of melanoidins, which are a recalcitrant pigment produced by the Maillard reaction between amino and carbonyl groups present

in the organic matter [44,46]. The EO pre-treatment caused a decolourisation of the sample which was also associated with the decrease in organic carbon.

The concentration of ethanol and polyphenols decreased when increasing the duration of EO treatment (see Figure 2c). Ethanol concentration experienced a linear decrease with the increment in the treatment time ($39 - 10.1 \times t$; $R^2 = 0.814$), while in the case of polyphenols the behaviour is better described by a logarithmic approximation ($23.6 - 5.13 \times Ln(t)$; $R^2 = 0.989$) indicating a marked effect in the initial times. WL analysed in this study has a high content of polyphenols that can hinder AD. The EO achieved a reduction between 25% to 60% in the elimination of this polyphenols (Figure 2d), which seems appropriate as a pre-treatment option when considering the subsequent AD of WL.

The experiments of Candia-Onfray et al. [47] treating winery wastewater using BDD electrodes reached almost complete mineralisation of organic matter when applying 20, 40 and 60 mA cm^{-2} at 0.1 L of aqueous solutions containing 3490 mg L^{-1} of COD. In the present study, current density was lower (18.8 mA cm^{-2}) and WL sample was characterised by a higher content of organic matter and lower conductivity (see Table 2), which reduced the EO performance when using the BDD cell.

The aforementioned increase in VFAs and acetic acid contents (Table 4 and Figure 3) could be explained assuming that EO could promote the conversion of complex organic molecules into simpler species. Three types of short-chain VFAs were measured (acetic, propionic and butyric acids), with the highest concentration corresponding to acetic acid. This acid is a type of substrate that can be directly assimilated and transformed into methane through methanogenesis, thus the observed enhancement on its production could be directly associated with an expected increase in methane production due to EO. Nevertheless, the content of acetic acid must be within values below those capable of causing inhibition of the digestion process due to the activity of methanogens could be reduced by the VFA accumulation [48]. In most cases, moderate inhibition was reported at acetic acid concentrations of 900–1800 mg L^{-1} for initial propionic acid concentrations in the range between 740–1850 mg L^{-1} [49].

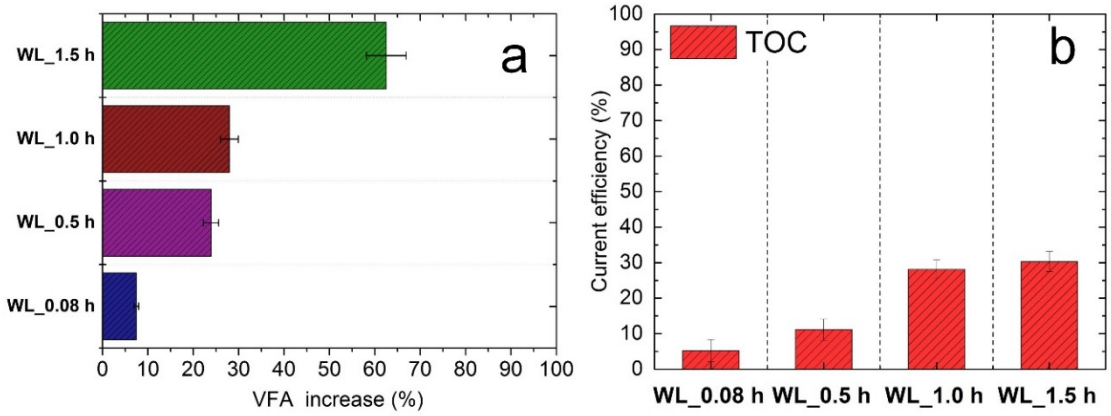

Figure 3. Effects of electro-oxidation pre-treatment on: (**a**) volatile fatty acids (VFAs) and (**b**) current efficiency calculated using the decrease in total organic carbon (TOC) values as a base.

The current efficiency (CE) values (Figure 3b) were approximately 30%. The pronounced increase of CE values with the working time indicated a better removal of organic matter, which was related to the application of higher current density when testing values from 11 to 18 mA cm^{-2}. The current density is directly associated with the number of hydroxyl radicals generated (capable of reacting with organic matter). These parameters, along with the applied treatment time, are well-known factors to improve EO.

3.2. *Effect of EO Pre-Treatments on AD*

The results of AD tests (Figure 4) demonstrated a positive effect of EO on biogas, considering the final gas volumes and production rates. Final biogas volume measured for WL (around 0.18 L kg^{-1} VS)

was not far from literature values [9]. EO improved biogas final volumes after 1 h of EO, and up to about 330 L kg^{-1} VS after 1.5 h of EO. The average methane content in all digesters was 58%.

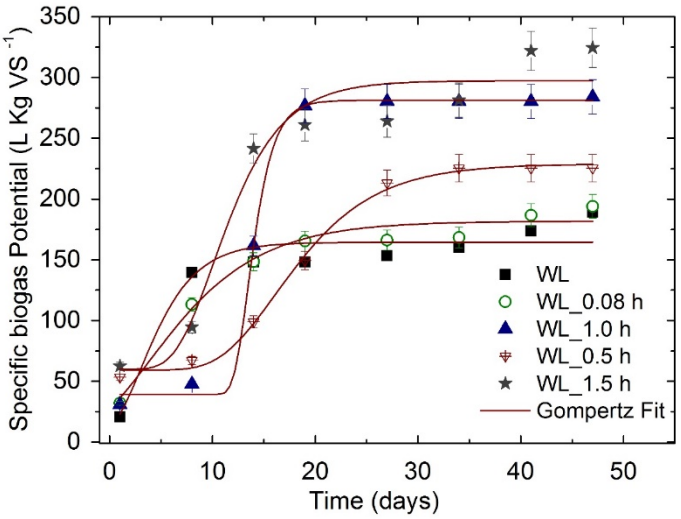

Figure 4. Cumulative specific biogas potential of AD tests after electro-oxidation pre-treatments.

The transformations involving the organic matter during the EO process allowed an effective assimilation of the substrate by microorganisms. The results regarding the reduction or removal of the different parameters may not be considered adequate in terms of the individual performance of the EO process (Figure 2; Table 4). However, the elimination/decrease of complex substances as melanoidins, polyphenols and alcohols are adequate to allow further degradation of WL in a subsequent stage of AD.

Nevertheless, methane production was unstable in all reactors and particularly at the beginning of AD. A lag phase (Table 5) that varied from 0.033 to 7.38 days was observed in the pre-treated WL samples, which could be explained considering the high concentration of VFAs and polyphenols in WL (Table 2).

Table 5. Kinetic adjustment results of AD tests after electro-oxidation pre-treatments.

		Modified Gompertz				
		Wine lees	**WL_0.08 h**	**WL_0.5 h**	**WL_1.0 h**	**WL_1.5 h**
Biogas potential [m^3 kg^{-1} VS]	P	0.164	0.181	0.241	0.25	0.237
Maximum biogas production rate [m^3 kg^{-1} VS d^{-1}]	R_{max}	0.02	0.043	0.009	0.007	0.032
Lag phase [days]	λ	0.033	2.34	4.02	6.87	7.38
Coefficient of determination	R^2	0.9	0.956	0.998	0.997	0.92
Standard error of estimates	SSE	0.005	0.006	0.009	0.008	0.009

The EO pre-treatment caused partial oxidation of the organic material and the complex molecules present in the sample, melanoidins, polyphenols and higher organic acids were oxidized and transformed into simpler molecules such as acetic acid when the pre-treatment was prolonged. The EO treatment was more effective for the longest time of pre-treatment application (see Figures 2 and 3 and Table 4). Nevertheless, acidic media (high concentration of VFA), can cause a slow start for the anaerobic digestion, a higher concentration of VFA (acetic, Figure 3) in samples corresponded to extended lag phases.

VFAs are inhibitors of the AD process when their concentrations exceed 50–250 mg L^{-1} [50]. Furthermore, the form in which the acid is present in the solution (as dissociated or undissociated form, which in turn depends on the pH of the solution) is a key factor in causing the inhibition

phenomena. Undissociated VFAs can freely enter the cytoplasm through the membrane and be metabolised, whereas the cell membrane remains impermeable to the dissociated ion [51]. Although in this study the AD tests started with a relevant concentration of VFAs (Tables 2 and 4), having an important impact over the lag phase in the pre-treated samples, pH was adjusted to 7.5 (Section 2.4) and no inhibition was observed in methane production after EO pre-treatments (Figure 4).

Different studies found that the presence of phenolics substances in wine production liquid wastes from 60 to 667 mg L^{-1} may result in low biogas production and instability [52–54]. The concentrations of polyphenols in the digestion liqueur obtained in this study were 58 to 20 mg L^{-1} of gallic acid lower to the previously mentioned thresholds.

The values of VFA obtained from the different digestion tests would cause acidification of the medium (expecting a pH < 5) and as consequence, the methanogens would have been inhibited. However, the initial addition of sodium bicarbonate neutralised the acid nature of the medium [55] and kept pH values between 6.8 to 7.5 allowing the process to continue.

3.3. *Effect of Biochar Addition on AD*

The results of AD tests performed adding biochar (Figure 5 and Table 6) were as follows. The cumulative specific biogas production from WL was analogous to the results previously obtained (Figure 4). No methane was measured during AD tests supplemented with BC or PS. A one-way analysis of variance (ANOVA) demonstrated that there was not any significant difference between the specific biogas productions of WL in the presence of BC and PS and WL alone ($F(2,6) = 0.146$, $p = 0.87$). It was observed that for recalcitrant materials and other complex organic wastes, as it could be the case of WL, an extra disintegration process becomes necessary before AD, either as a pre-treatment or during fermentation. Considering the mass balance of TS and VS (Figure 6a), the solids removal during AD seems scarce, and the amount of total VFAs (Figure 6c) far above the critical values cited above [50].

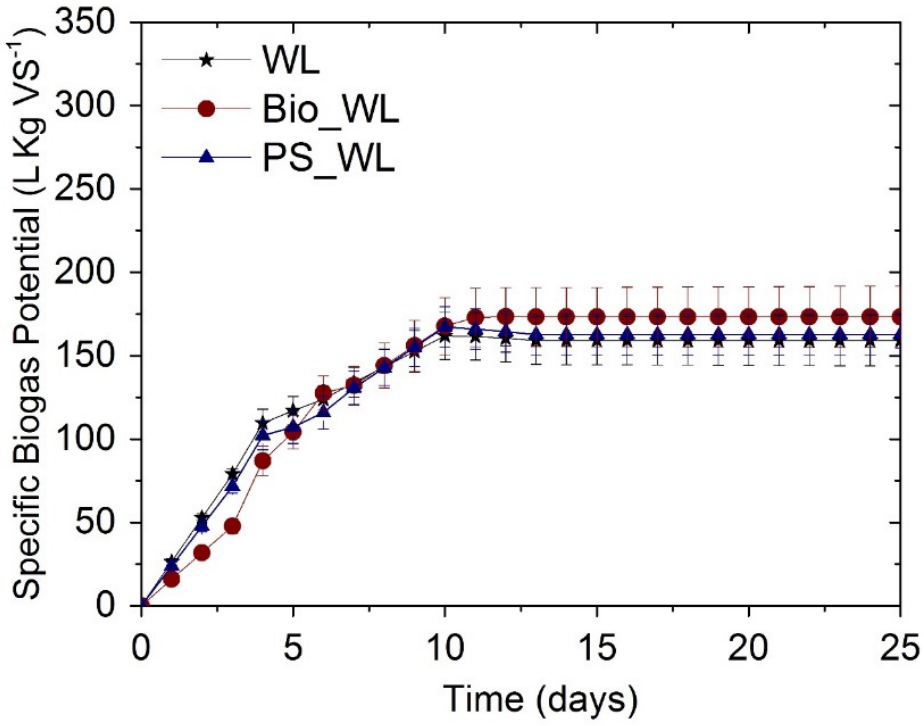

Figure 5. Cumulative specific biogas potential measured for wine lees with biochar (Bio_WL) and pumice stone (PS_WL) and comparison with wine lees (WL).

Table 6. Kinetic parameters obtained from fitted data to the Gompertz model. Results from batch AD tests of WL with biochar and pumice stone.

		Modified Gompertz		
		Wine Lees	**Biochar**	**Pumice Stone**
Biogas potential [m^3 $kgVS^{-1}$]	P	0.161	0.190	0.170
Maximum biogas production rate [m^3 $kgVS^{-1}$ d^{-1}]	R_{max}	0.027	0.031	0.023
Lag phase [days]	λ	0.153	1.057	0.100
Coefficient of determination	R^2	0.922	0.973	0.923
Standard error of estimates	SSE	0.007	0.006	0.009

Some hypotheses (hp) could be formulated considering the results of AD test when digesting WL with either BC or PS:

(hp1) The fermentation of a highly biodegradable substrate rich in inhibitors promoted the production of other inhibitors during the early stages of the AD process (Figure 3 and Table 4), resulting in low biogas production.

(hp2) A lower (than 1:1 value adopted in this study) substrate to inoculum ratio could eventually improve the stability of the system, specifically diluting inhibitory substances and adding a larger amount of active biomass.

(hp3) Despite BC addition, which was verified to be effective in the adsorption of inhibitors, the specific biogas production obtained in the first 4 days was lower for WL in the presence of BC than either for WL with PS addition or WL as sole component (Table 5). This may indicate that the addition of BC resulted in an initial decrease in biogas production, possibly because of CO_2 adsorption, and an increase in the lag phase of one order of magnitude.

(hp4) Comparing pH values before and after AD tests (Figure 6b), it was observed that any pH drop occurred during the preliminary phases of the AD process was associated with an initial increase of an efficient buffering effect due to the alkalinity added to the system before the tests.

(hp5) Specific biogas production measured from WL and PS supplemented digesters seemed analogous to the trend observed for WL alone (Table 6). PS was used as inert support for microbial attachment and growth (see Section 2.3). In this work, the presence of PS did not seem to play any positive role at the dose evaluated.

(hp6) The adsorption of BC was not selective and it is possible that nutrients and useful metabolites such as nitrogen source were also adsorbed. The addition of BC to the AD of WL presented an analogous trend compared to WL alone. However, the increment in VFAs (Figure 6c) may indicate that degradation was slightly enhanced, but this in turn led to higher concentration of VFA. VFAs, in addition to inhibitors already present in WL, may be responsible for the delay in biogas production observed in this work. It should be also mentioned that WL considered in this work was sampled in July, about 10 months after grapes pressing and the beginning of fermentation processes happening in wine-making (e.g., readily biodegradable organic compounds were already fermented, leaving behind less biodegradable and more acidic compounds).

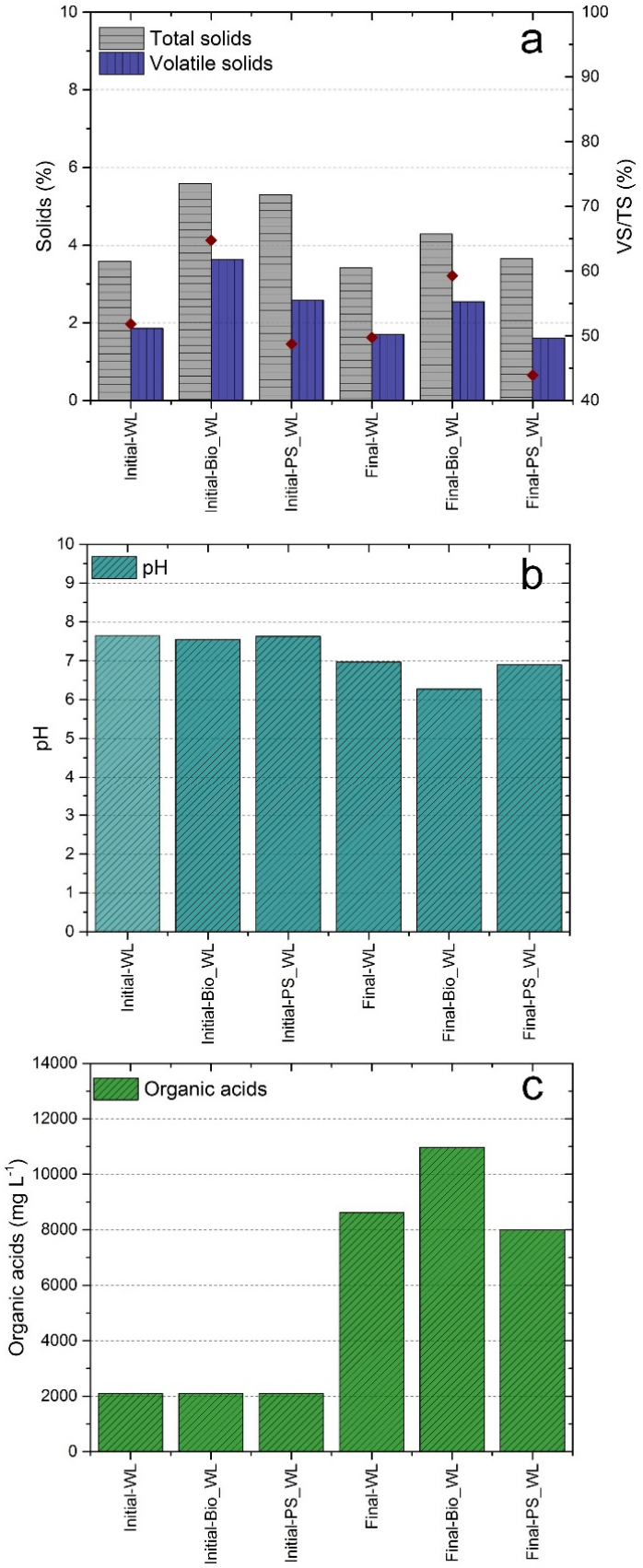

Figure 6. Comparison of physico-chemical characteristics of substrates (initial values) and digestates (final values): (**a**) TS and VS (%) and VS/TS (% of TS); (**b**) initial and final pH values; (**c**) total VFAs (mg L^{-1}).

4. Conclusions

On the grounds of the results obtained from this work, some conclusions could be made in an attempt to answer the RQs mentioned in Section 1. Firstly, AD seemed an appropriate treatment process for the valorization of WL if the application of EO as pre-treatment is considered. Taking into account the complex nature of WL, a strong oxidation pre-treatment appears crucial to improve biogas production and enhance methanogenesis. Further research is necessary to investigate in detail the operating conditions to assure stable methane production and to evaluate energy needs associated with the coupled configuration regarding pre-treatment and subsequent digestion. Secondly, in the experimental conditions explored, BC did not exhibit any significant benefit, as it is usually reported in the literature. In fact, the mere effect of BC as physical support for biomass growth was not observed. WL are biodegradable, but with a high content of inhibitors and also strongly acidic. It is necessary to explore operative conditions that could prevent any overload of AD and effectively improve methanogenesis.

Author Contributions: Conceptualization, methodology, supervision, manuscript writing and review: E.J.M. and S.F.; methodology, writing—review and editing: X.G.; experimental activity, data elaboration, original draft writing: C.B.A.S. and M.C. All authors have read and agreed to the published version of the manuscript.

Funding: Cristian B. Arenas would like to thank The Ministry of Economy and Competitiveness for fellowship BES-2016-078329. The authors want to thank the Project 0688_BIOVINO_6_E that has been carried out with the financial assistance of the FEDER and the POCTEP.

Acknowledgments: Gratefully acknowledge UK Biochar Research Centre at the University of Edinburgh, United Kingdom for supplying the biochar used in the research.

Conflicts of Interest: The authors declare no conflict of interest.

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