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Suitability of olive oil washing water as an electron donor in a feed batch operating bio-electrochemical system

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SUMMARY: Olive oil washing water derived from the two-phase manufacturing process was assessed as an electron donor in a bio-electrochemical system (BES) operating at 35 ºC. Start-up was carried out by using acetate as a substrate for the BES, reaching a potential of around +680 mV. After day 54, BES was fed with olive oil washing water. The degradation of olive oil washing water in the BES generated a maximum voltage potential of around +520 mV and a Chemical Oxygen Demand (COD) removal efficiency of 41%. However, subsequent loads produced a decrease in the COD removal, while current and power density diminished greatly. The deterioration of these parameters could be a consequence of the accumulation of recalcitrant or inhibitory compounds, such as phenols. These results demonstrated that the use of olive oil washing water as an electron donor in a BES is feasible, although it has to be further investigated in order to make it more suitable for a real application.

KEYWORDS*: Bio-electrochemical system; COD removal; Electricity generation; Electron donor; Olive oil washing waters*

RESUMEN: ***Idoneidad del agua de lavado de aceites de oliva como donador de electrones en un sistema alimentado por lotes bioelectroquímico.*** El agua de lavado del aceite de oliva procedente del proceso de elaboración en dos fases fue utilizada como donador de electrones en un Sistema Bioelectroquímico (BES) operado a 35ºC. Se realizó una etapa de arranque del sistema mediante alimentación con acetato, alcanzando un potencial de referencia de +680 mV. Tras 54 días, el sistema se alimentó con agua de lavado de aceite, generando un potencial máximo de +520 mV y una eliminación de materia del 41%, en demanda química de oxígeno. Sin embargo, cargas subsecuentes conllevaron una bajada en la eliminación de materia, mientras que la densidad de corriente y de potencia disminuyeron ostensiblemente. El empeoramiento de estos parámetros puede deberse a la acumulación de compuestos recalcitrantes o inhibidores, como fenoles. Por tanto, el uso del agua de lavado de aceite de oliva en un BES es factible, aunque es necesario llevar a cabo nuevas investigaciones que hagan más atractiva su aplicación a escala real.

PALABRAS CLAVE*: Agua del lavado del aceite de oliva; Donador de electrones; Eliminación de materia; Generación de electricidad; Sistema bioelectroquímico*

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1. INTRODUCTION

Circular bioeconomy is nowadays becoming a major issue for sustainable development. In the olive oil sector, circular economy includes the reduction of the pollution footprint of the whole process and the valorization of the different waste streams. 2.2 million tons of olive oil are produced annually in the European Union, which amounts to more than 75% of worldwide production. From the produced 2.2 million tons, 1.2 million tons are produced in Spain, mainly in the Andalusia region (IOOC, 2016). The two-phase olive oil extraction process is the main technology for olive oil production in this region (Rincon *et al.,* 2012). In this manufacturing process, around 0.55 m^3 of wastewater per ton of processed olives are produced (Borja, *et al.,* 2006). 0.15 m^3 out of the 0.55 m^3 per ton of processed olives counted for wash waters derived from the washing of olives. Olive washing water can be employed for irrigation given that its content in organic matter is rather low. Most wash waters from the initial washing of olives comply with the Andalusian regional regulation for irrigation purposes, i.e. pH = 6–9; Suspended solids < 600 ppm; BOD_5 < 2000 ppm; Chemical Oxygen Demand (COD) < 2500 ppm; limitation for spreading on the terrain: 30 m^3/Ha every 7 days (Decree 4/2011 of 11 January 2011, on the regulation of the use of effluents from olive mills for irrigation purposes).

0.4 m^3 out of the 0.55 m^3 per ton of processed olives counted for wash waters derived from the purification of olive oil, which is around 480,000 m^3 of wastewater per year only in Spain (Borja, *et al.,* 2006). Wash waters generated during the purification of olive oil usually present a much higher pollution potential than olive washing water and, therefore, they must be adequately managed (Balasundram *et al.,* 2006). Their COD values are higher than the Regulation limit values in all cases. The lack of reliable management of this residue represents an environmental challenge, not only for aquatic ecosystems but also for soil and atmosphere. One promising method for the valorization of wash waters generated during the purification of olive oil is the production of biogas. Pilot scale experiences have been already successfully tested (Hauptmeier *et al.,* 2016). Although biogas production is a very promising method, one constraint of this technology is the need for different upgrading methods in order to use the obtained biogas. Other alternative technologies of renewable energy production base on anaerobic fermentation are the so-called Bio-Electrochemical Systems (BESs) (Hernandez-Fernandez *et al.,* 2015). Typical BES configuration uses micro-organisms to catalyze an oxidation and reduction reaction at an anodic and cathodic electrode, respectively (Hernández-Fernández *et al.,* 2015). Oxidation of organic and inorganic electron donors occurs in the anode (Ter Heijne *et al.,* 2006). The organic waste material, such as olive oil washing water, can be used as an electron donor in this process. The anode and the cathode are connected to an electrical circuit where electricity can be harvested (Bajracharya *et al*., 2016). The use of microorganisms to catalyze the electrochemical oxidation of organics is highly attractive as it allows taking advantage of the versatility and resilience which bacteria exert. Responsible for the process are specific bacterial species, the so-called "exoelectrogens", mainly belonging to the gamma- and delta-subgroups of Proteobacteria (Sciarria *et al.,* 2013). The potential of BESs is enormous since this technology has important operational and functional advantages over the current technologies used for generating energy and valuable compounds from organic matter (Hernández-Fernández *et al.,* 2015). Furthermore, BESs do not require a gas treatment because the off-gases of these devices are enriched in CO_2 and normally have no useful energy content.

BESs have been validated at lab-scale with simple organic substrates, pure cultures, and very controlled experimental conditions. Simply organic substrates such as acetate and volatile fatty acids have been widely employed (Clauwaert *et al*., 2007; Catal *et al*., 2008). During recent years, the improvement in the design of BESs has tremendously increased electric generation (Yang *et al*., 2015). Employment of complex substrates as an electron donor is not really extended and could represent an interesting management alternative that should be properly evaluated. The implementation of BESs for olive oil mill washing water as an electron donor is highly promising given that there is no need for post-treatment units and the produced electrons could be used for direct energy or other reduced valuable compound production directly in the BES unit.

The aim of this research was to study the suitability of olive oil washing water as an electron donor in a BES. At the same time, the efficiency of the process in terms of COD removal and electricity production was also tested.

2. MATERIALS AND METHODS

2.1. Olive oil washing water

Wash water from the secondary centrifuge generated during the purification of virgin olive oil (two-phase manufacturing system) was collected from the Experimental Olive Oil Factory located in the "Instituto de la Grasa (CSIC)" of Sevilla, Spain. Prior to its characterization and use, olive oil washing water was preserved under freeze conditions (-4 ºC) to avoid undesirable fermentation processes. The main characteristics of the used olive oil washing water are shown in Table 1.

2.2. BES set-up

The BES consisted of two Plexiglas plates with a single-flow channel, two electrodes, and two Plexiglas support plates (Figure 1). The two Plexiglas plates with a flow channel were separated by a cation exchange membrane (Fumasep FKB, Fumatech, St. Ingbert, Germany). The other side of the flow channel faced the electrode. The anode and cathode electrodes were made of flat graphite (MR200, gas tight impregnated, from Müller & Rössner GmbH & Co., Troisdorf, Germany).

TABLE 1. Physicochemical characterization of olive oil washing water

pH		5.65 ± 0.05
Alkalinity	(mg $CaCO_3$/L)	300 ± 50
COD	(mg O_2/L)	8435 ± 420
sCOD	(mg O_2/L)	2195 ± 30
VS	(mg/L)	7210 ± 290
FS	(mg/L)	455 ± 60
Total Phenols	(mg Gallic acid/L)	255 ± 7
Soluble Phenols	(mg Gallic acid/L)	211 ± 4

±, *Standard deviation*.

The surface area of the flow channel and thus, the projected surface area of the electrodes in contact with the solution was 22 cm^2. The volume of the flow channel was 33 mL (11.2 cm long x 2.0 cm width x 1.5 cm high) in each the cathode and anode compartment. The voltage of the system was acquired every 10 minutes via a data acquisition card (NI USB-6009) connected to a computer. The BES was kept at a temperature of 35 ºC. The anode compartment of the BES was connected in a closed system to a 1-liter bottle, where inoculum and used substrate were placed. The cathode compartment of the BES was connected in a closed system to a 1-liter bottle, where the catholyte was placed.

2.3. BES operation

The inoculum placed in the anode was obtained from an industrial anaerobic reactor (UASB) treating brewery wastewater and operating at mesophilic conditions (35 ºC). The main characteristics of this anaerobic inoculum were: pH: 7.5; Total Solids (TS): 69 g/L; and Volatile Solids (VS): 45 g/L. The BES was run in batch mode and substrate was batch-fed to the anode every time the current and the power densities were almost zero. During the first 36 days of operation, The BES was fed with

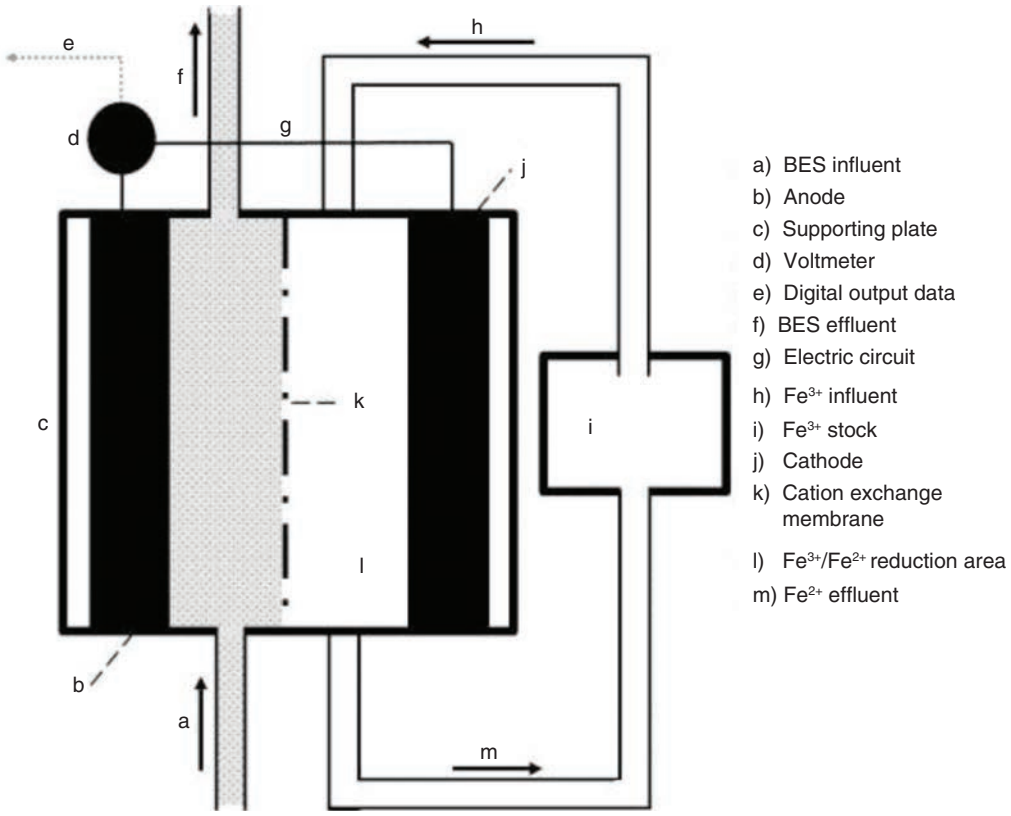

FIGURE 1. Bio-electrochemical system used.

acetate in the anode compartment. The catholyte was composed of a phosphate buffer solution and flushed with air for 36 days. On day 36 of operation, the catholyte was replaced by a solution of Fe(III) $[CN]_6^{3-}$ (0.050M) and phosphate buffer (reduction of Fe(III)$[CN]_6^{3-}$ to Fe(II)$[CN]_6^{4-}$). From day 36 to day 54, acetate was still fed to the anode compartment. On day 54, acetate was no longer fed to the anode compartment but olive oil washing water till the end of the experiment at day 63.

2.4. Measured and calculated parameters during BES operation

The current density I (A/m^2) was calculated from the measured voltage potential (V) of the BES, the used resistance (R), whose value was 500 Ω (ohms) throughout the experiment and the projected surface area of the electrode (A_{elec}) by using equation (1). Power density E (W/m^2) was calculated by using equation (2):

$$I = V/(R \cdot A_{elec}) \quad (1)$$

$$E = V \cdot I \quad (2)$$

The Coulombic Efficiency (CE) was described as the percentage of electrons recovered from organic matter versus the theoretical maximum whereby all electrons are used for electricity production (Tee *et al.*, 2017). Therefore, CE was calculated by using equation 3:

$$CE(\%) = Ca/Ct \times 100 \quad (3)$$

where Ca is the total coulombs calculated by integrating the current over time, and Ct is the theoretical amount of coulombs available from the oxidation of olive oil washing water.

2.5. Chemical analyses

All chemical analyses were performed according to the Standard Methods of APHA (APHA, 1998). The following parameters were analyzed: Chemical Oxygen Demand (COD), Soluble Chemical Oxygen Demand (sCOD) (method 5220D), Total Solids (TS), Fixed Solids (FS), and Volatile Solids (VS). pH and alkalinity were determined by using a pH-meter model Crison 20 Basic. Total and soluble phenols were quantified by spectrophotometry through the Folin-Ciocalteu method with a calibration curve of gallic acid (García *et al.*, 2016).

3. RESULTS AND DISCUSSION

3.1. Start-up

During the start-up, BES was fed with 1 g/L of acetate each 4-day period with the aim of promoting the formation of the microbial community in the anode. Figure 2 shows the voltage potential throughout the experimental time and the different operational conditions. During the first 35-day period, the voltage potential remained constant at a value of +41.4 ± 6.3 mV. At day 35, voltage potential increased up to +210.3 ± 13.7 mV (Figure 2). This potential was reached by using an air fed cathode, with oxygen as the electron acceptor. The increase in the voltage potential indicated the development of the microbial community in the anode.

At day 36, the air-fed cathode was substituted by a ferric/ferrous iron cathode. The substitution was carried out because a ferric/ferrous iron cathode allows for obtaining a higher standard potential

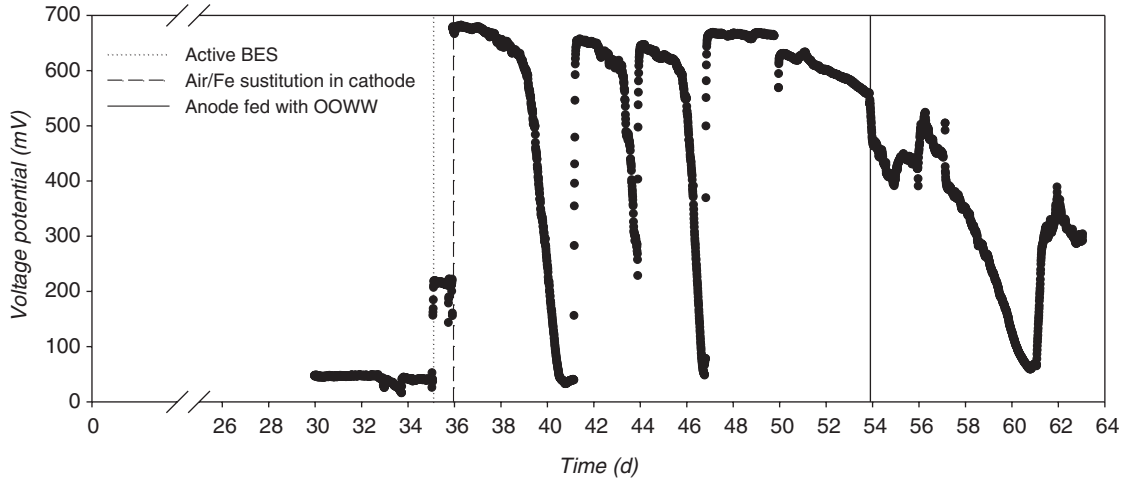

FIGURE 2. The voltage potential of the BES system during the experimental time.

than the air cathode, i.e. +770 mV vs. a normal hydrogen electrode (Uría *et al*., 2012). This kind of cathode presents other advantages compared to the air cathode such as fast reaction at carbon electrodes (Uría *et al*., 2012, Ter Heijne *et al.,* 2006).

As can be seen in Figure 2, the substitution of the air cathode by a ferric/ferrous iron cathode resulted in a rapid increase in the voltage potential, which reached a maximum potential of +680 mV. This value was very similar to the standard potential for the ferric/ferrous iron cathode, indicating that the microbial film in the BES was properly developed. From day 36 to day 54, each feed batch of acetate resulted in an instantaneous increase in the voltage potential until achieving a value close to +680 mV. After the initial increase, the voltage potential slightly decreased during the 2-day period. A drastic drop in the voltage potential was observed around 4 days after the feed batch, which decreased to +40 mV (Figure 2). This decrease was a consequence of the substrate limitation in the BES, given that the acetate is a readily digestible substrate which requires a very short time for its degradation. Voltage potential was recovered to the reference value after each feed to the BES. After a 54-day period, the behavior of the system was kept under stable and reproducible conditions, and the reference value of the voltage potential was kept virtually constant (Figure 2).

3.2. Olive oil washing water feeding

The electrochemical active anode was fed with olive oil washing water on days 54 and 61. This substrate allowed for reaching a maximum voltage potential of +520 mV, i.e. 76.4% of the maximum voltage potential determined for acetate degradation (Figure 2). However, voltage potential reached lower values after the subsequent load of olive oil washing water on day 61, i.e. around +390 mV (Figure 2). After the addition of olive oil washing water at day 54, COD decreased during the time from 2600 ± 50 mg O_2/L on day 54 to 1535 ± 50 mg O_2/L on day 61 (41% COD removal). During these 7 days, two different periods can be observed. One period from day 54 to day 57, where current and power density were stable (Figure 3A) and COD was effectively degraded (Figure 3B). In the second period, from day 57 to day 61, COD gradually decayed (Figure 3B), while current and power density decreased greatly (Figure 3A). At days 54-57, most likely only the most readily digestible organic matter was degraded by the microorganisms present in the anode supporting electricity production. While at the second period, the most recalcitrant organic matter did not support electricity production.

A maximum COD removal of 43%, very similar to that achieved in the present work (41%), was obtained by Sonowane *et al*. (2013) in a multi-electrode MFC (multiple anodes acting as a baffle) using diluted distillery wastewater (pH: 7.8; COD: 2303 mg/L; total solids: 35.2 mg/L) in three batches at ambient conditions. Maximum COD and Biological Oxygen Demand (BOD_5) removal efficiencies of 65% and 50% respectively were reported in a single-chamber air cathode BES with platinum anode treating classical olive mill wastewater diluted with domestic wastewater at the ratio of 1:14 (COD: 4300 mg/L). DNA-fingerprinting showed the high bacterial diversity and the presence of exoelectrogenic bacteria on anodes, such as *Geobacter spp* (Sciarria *et al.,* 2013).

In the present study, a feed batch cycle of 4 days was enough for acetate degradation, however, only 41% COD degradation of olive oil washing water was achieved after a 7-day cycle. Maximum dissolved organic carbon (DOC) removal of 90% in feed batch cycles of 15 days was achieved in a dual chambered BES operated in batch mode with platinum and mixed metal oxide titanium (Ti-TiO_2) with initial DOC values of 3600 mg/L. A decrease in feed batch cycles from 5 to 15 days adversely affected BES performance (Cirik, 2014). In the same way, a COD removal efficiency of 79% was reported in a dual chambered BES operating with wastewater with sulfate at a COD/sulfate ratio of 0.8 and feed batch cycles of 2 days (Ghangrekar *et al*., 2010).

Low power densities are usually reported when real wastewaters or severe environmental conditions are used. Lower power density values (65.82 mW/m^2) than that achieved in the present research (Figure 3A) were reported by Zhang *et al*. (2013) using diluted molasses in a two-chamber BES with an external resistance of 1000 Ω. Maximum power densities as low as 17.6 mW/m^2 were reported in two-chamber BES treating mining process wastewaters containing tetrathionate at pH below 2.5 with ferric iron as the terminal cathodic electron acceptor (Sulonen *et al.,* 2014). On the contrary, maximum power density values as high as 1180 mW/m^2 were achieved in MFC treating wastewater produced in a bio-refinery process (COD: 5.3 g/L) containing residual sugars, 5-furfural, phenolics, and other pretreatment and fermentation by-products (Borole *et al.,* 2013). In this case, power density increased with loading reaching the above-mentioned maximum value at COD of 5.3 g/L (8% dilution) but decreased thereafter. It was demonstrated that excessive loading led to poor electrogenic performance (Borole *et al.,* 2013).

Figure 3C shows the variation in the CE percentage during the experimental time, ranging from 3.0% to 0.5%. These results are consistent with those obtained by Koók *et al*. (2016) and Capodaglio *et al.* (2015) treating the liquid fraction of pressed municipal solid waste and urban wastewaters, respectively. However, other authors, using synthetic waters, obtained higher percentages (Aelterman *et al.*, 2008; Sleutels *et al*., 2009). The differences in the

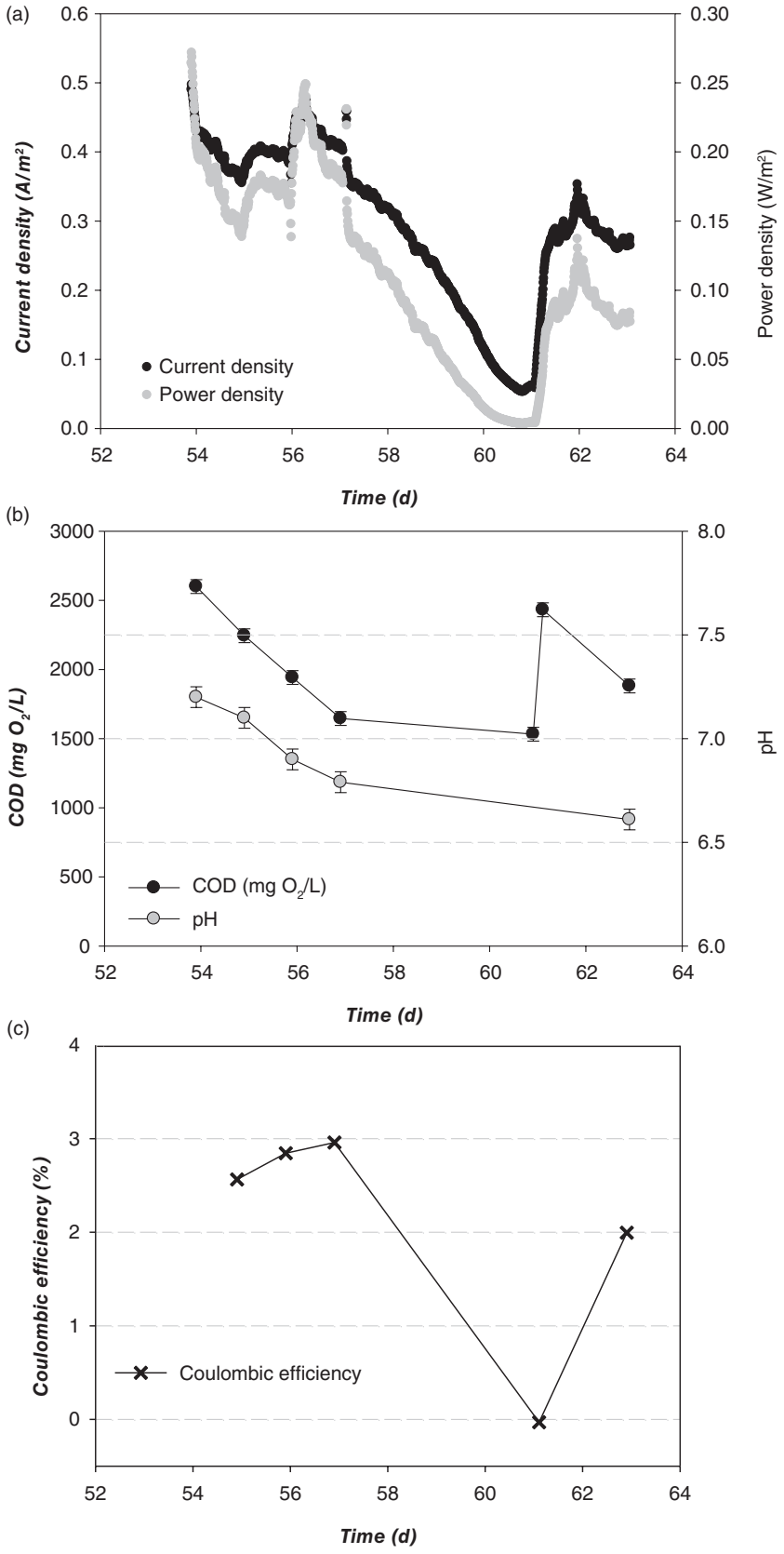

FIGURE 3. **A)** Current density and power density over time. **B)** pH and COD over time. **C)** Coulombic efficiency over time.

CE values in the reported studies can be attributed to the reactor configuration, source of inoculum, external resistance used, etc. The low CE is a general issue in BES using real wastewater. The presence of inhibitory compounds, such as phenols, limits the COD removal as observed in the residual COD of the present study (Figure 3B). These phenolic compounds are known to show antimicrobial properties (Acar *et al*., 1992; Khoufi *et al.,* 2004; Chen *et al.,* 2010; Mohamed *et al.,* 2010).

4. CONCLUSIONS

This study demonstrates for the first time that olive oil washing water from the two-phase manufacturing process can be used as an electron donor in BES. Although electricity generation using these wastewaters is feasible, recalcitrant organic matter degradation has to be further investigated in order to make it more suitable for a real application.

BES could be a possible solution to the treatment of olive mill liquid residues. The possibility of treating wastewater and also the possibility of generating energy and/or valuable compounds from organic matter on a small scale as BES offer might make them a highly promising technology for olive oil factories.

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