Treatment of two-phase olive mill waste and degradation of phenolic compounds with simultaneous bio-electrochemical conversion of CO_2 to CH_4 using a Microbial Electrolysis Cell

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Abstract This study deals with the treatment of a potent two-phase olive mill waste (TPOMW) through the degradation of its phenolic content, with simultaneous bio-electrochemical reduction of CO_2 to CH_4, using a dual-chamber Microbial Electrolysis Cell (MEC). The MEC operated for 120 days and the effects of different dilutions (1:10, 1:5 and no dilution) and applied potentials (0.5 V and 1 V) on its performance were studied. The results showed that decreasing the dilution (from 1:10 to 1:5 and to no dilution) led to an increase of the COD removal (from 74%, to 77% and to 87%, respectively), of the total phenolic content removal (from 73%, to 76% and to 79%, respectively), as well as of the produced CH_4 (from 0.08, to 0.48 and to 2.33 mmols, respectively). Increasing the applied potential (from 0.5 V to 1 V), while the TPOMW was employed in the anode with no dilution, resulted in further increase of both the COD and the total phenolic content removal to 91%, while the produced CH_4 further increased to 2.88 mmols. The results indicate that the MEC technology can be potentially exploited for the treatment of the potent TPOMW and produce CH_4 as a waste-to-energy source.

Keywords: MEC; TPOMW; phenols; electromethanogenesis; applied potential

1. Introduction

The olive oil industry is a highly concentrated sector, 97% of which is traced around the Mediterranean and the annual waste generation is expected to surpass 3 million tons (Dermeche et al., 2013). The two-phase olive mill waste (TPOMW), originating from the two-phase olive oil extraction procedure, is the most potent by-product of the olive oil sector (Sánchez-Sánchez et al., 2020). Its high organic content is accompanied by low biodegradability, attributed to the presence of toxic phenolic compounds, which persist in the environment and comprise a risk for the environment and human health (Fleyfel et al., 2022).

Bioelectrochemical Systems (BES) are a promising technology for the valorization of powerful contaminants, while contributing to the energy recovery of the process (Modestra et al., 2022). Specifically, the Microbial Electrolysis Cell (MEC) constitutes an innovative bioreactor, since a small potential can be applied in order to both improve the bio-degradability of toxic contaminants, such as the phenols present in the TPOMW, as well as to achieve a sustainable waste-to-energy conversion towards an added-value by-product (Koul et al., 2022).

In this context, the scope of the present study is to assess the valorization of the TPOMW in the anodic compartment of a dual-chamber MEC, along with the bio-electrochemical conversion of CO_2 to CH_4 in the cathodic compartment. This was accomplished through monitoring the generated current of the MEC, along with the degradation of the TPOMW organic constituents, under various operating conditions. The proposed system has the potential to effectively treat the potent waste, while producing a added-value product which contributes to the energy recovery of the process.

2. Materials and Methods

2.1. MEC setup, inoculation and the TPOMW substrate

For this work, a dual-chamber MEC was constructed, as described elsewhere (Kanellos et al., 2022). The two chambers were separated using a Proton Exchange Membrane – (PEM) (Nafion ® 115, DuPont), with an area of 25 cm^2. Carbon felt was used for both the anodic and cathodic electrodes, with dimensions of 5 cm x 5 cm for the anode, while the cathode was comprised of a carbon felt cube with dimensions of 5 cm x 5 cm x 2 cm. A titanium wire connected the electrodes with the external circuit. The anodic compartment volume was 50 mL, the cathodic compartment volume was 100 mL and the cathode headspace was 60 mL. The anodic compartment was equipped with a sampling port and the cathodic compartment with a sampling point for gas detection. The system was placed in a temperature controlled box (30 ºC) and operated in batch mode.

Anaerobic Sludge, obtained from the Municipal Wastewater Treatment Plant of Metamorphosis, in Attica, Greece was used as the inoculum (10 % v / v) for both the anode and the cathode during the MEC start-up. During the acclimation period, the anolyte also contained phosphate buffer (3.67 g / L NaH_2PO_4 and 3.45 g / L Na_2HPO_4), potassium chloride (0.16 g / L KCl), sodium bicarbonate (5 g / L $NaHCO_3$), trace elements (1% v / v) and glucose (1.5 gCOD / L), as described elsewhere (Skiadas and Lyberatos, 1999). The catholyte contained phosphate buffer (3.67 g / L NaH_2PO_4 and 3.45 g / L Na_2HPO_4), potassium chloride (0.16 g / L KCl), sodium bicarbonate (2 g / L $NaHCO_3$) and trace elements (1% v / v). The

acclimation period was considered completed when stable current generation and methane production were observed. After the acclimation period, the electrolytes were incorporated in the cell as previously described, however without further addition of sludge inoculum. Following the stable operation of the cell with synthetic media, the same synthetic medium was used as the catholyte, while the TPOMW was introduced in the bio-anode.

The characteristics of the TPOMW, obtained from an olive oil mill in Pallini, Greece, were: pH 4.7; conductivity 9.85 mS / cm; soluble COD 50 gO_2 / L; total COD 82 gO_2 / L; total phenolic content 3.1 eq_g_{Gallic_Acid} / L; acetic acid 6.2 g / L; propionic acid 1.5 g / L; butyric acid 2 g / L; iso-butyric acid 1.1 g / L; valeric acid 0.2 g / L; iso-valeric acid 0.5 g / L; total solids (TS) 37 g / L; volatile solids (VS) 25 g / L; total suspended solids (TSS) 9 g / L; volatile suspended solids (VSS) 8 g / L; Total Kjeldahl Nitrogen (TKN) 565 mg / L; particulate organic carbon 17 gC / L.

2.2. MEC operation

The MEC operated in batch mode for 120 d, which were divided into 4 operation phases. The TPOMW substrate was filtered before all operation phases (Whatman GF/A glass microfiber filters 55 mm). Moreover, for each case, a NaOH solution (2 M, pH 14.2, conductivity 295 mS / cm) was added until the anolyte reached a pH of 6 - 6.2. The alkaline pretreatment was conducted in order for the TPOMW pH to reach values that would not affect the bacteria on the bio-anode. All experiments were carried out in batch mode, at a constant temperature of 30 ºC. During all operation phases, multiple batch cycles were performed in order for the obtained results to be repeatable and each batch cycle was considered completed once the generated current was reduced to low values. Following the filtration and the alkaline pretreatment, a series of different dilutions were tested during the first three phases. The dilutions tested were 1:10 (for 4 batch cycles), 1:5 (for 9 batch cycles) and no dilution (for 3 batch cycles) in order to deduce the optimal operating conditions for the MEC. During the fourth phase, the TPOMW was incorporated in the anode with no dilution (for 4 batch cycles), however the applied potential was increased from 0.5 V to 1 V. At the end of each batch cycle, the anolyte and catholyte were replaced by fresh feeds.

2.3. Analytical methods and calculations

The Chemical Oxygen Demand (COD) and the total and volatile solids content was quantified according to the standard methods for the Examination of Water and Wastewater (APHA/AWWA/WEF, 2012). The conductivity was measured by digital instrument WTW INOLAB. The total phenolic content was determined according to the Folin Ciocalteu method, as described elsewhere (Lamprou et al., 2020). The CO_2 to CH_4 conversion was quantified using a GC-TCD (SHIMADZU GC-2014). The VFAs were quantified using 1 ml of sample acidified with 30 µL of 20% H_2SO_4 and were analyzed via a GC-FID (SHIMADZU GC-2010 plus). An external power supply (DC PS-1502DD) was used to apply a constant voltage on the MEC in a two electrode configuration, while the resulting current was recorded every two minutes by (Agilent Keysight 34972A LXI Data Acquisition/Switch Unit).

The anodic Coulombic efficiency, which represents the proportion of the generated current and the obtainable electrons from the oxidation of organic matter (measured as COD reduction), as well as the cathodic Faradaic efficiency, which represents the proportion of the bio-electrochemically produced CH_4 from the generated current, were calculated as described elsewhere (Logan, 2008; Jansen et al., 2012),

The obtainable bio-energy surplus, based on CH_4 combustion, was determined by Eq. (1).

$$E\ (kJ) = c * 891 * Q_{CH_4} - E_{input} \qquad (1)$$

where E the bio-energy difference, c is an average combustion efficiency of 84% (Najmi and Arhosazani, 2006), 891 (kJ / mol) is the bio-methane standard enthalpy of combustion (NIST, 2021), Q_{CH4} (mol) is the produced CH_4 over the course of a MEC batch cycle and E_{input} (kJ) is the energy input through the form of potential application.

3. Results and Discussion

3.1. The two-phase olive mill waste valorization

Figure 1 depicts the generated current versus time of the MEC operation phases 1-4, with respect to the COD measurements performed at the bio-anode. Moreover, Table 1 shows the MEC operation parameters during all phases. The degree of dilution directly affected not only the initial COD content in the anolyte, but also the amount of NaOH addition and therefore the initial anolyte conductivity.

Effect of different TPOMW dilutions on MEC performance

Initially, during phase 1, four consecutive cycles were performed with a total duration of 21 d. During all the cycles, the generated current exhibited a current peak at approximately 1.5 mA, which was quickly reduced to low values within 2-3 d of the MEC operation, without maintaining a plateau. During the respective time, the initial COD (4.1 ± 0.3 gO_2 / L) was reduced to 1 ± 0.1 gO_2 / L. The total phenolic content exhibited an equivalent removal of 73%, as shown in Table 1, while the cumulative VFAs were 100% degraded. The CE during phase 1 was measured at 12%. During phase 2, nine consecutive cycles were performed, with a total duration of 39 d. The increased NaOH addition of 2% v / v, relative to phase 1 (1.75% v / v), led to an increased initial anolyte conductivity of 2.5 mS / cm (relative to 2 mS / cm for phase 1). During phase 2, the generated current exhibited a higher current peak (approximately 2 mA), which maintained a plateau for 2.5 d, before being reduced to low values. In addition, the initial COD (5.9 ± 1.5 gO_2 / L) was reduced to 1 ± 0.3 gO_2 / L over the course of each cycle. Regarding the nature of the organic constituents, the total phenolic content was reduced from 0.7 eq_g_{Gallic_Acid} / L initially to 0.2 eq_g_{Gallic_Acid} / L, while the cumulative VFAs were 100% degraded. The average anodic CE exhibited an increase of 18% relative to phase 1 (Table 1). Phase 3, which employed the TPOMW with no dilution, followed,
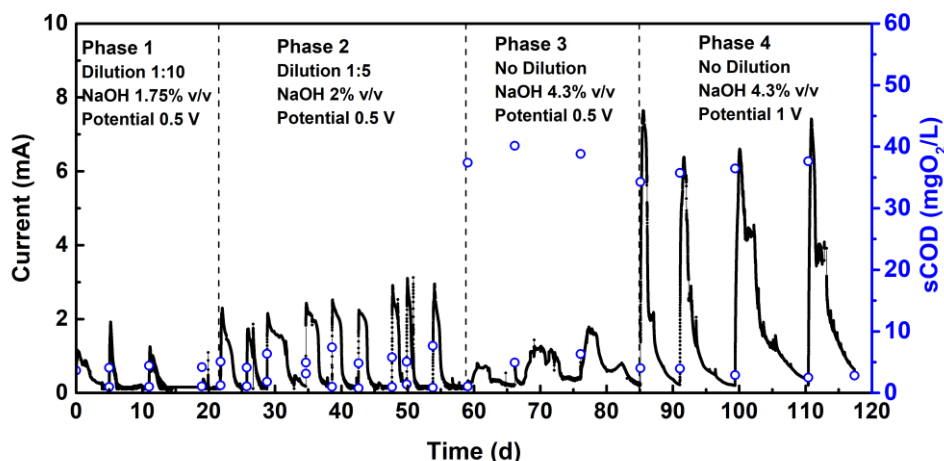
Figure 1. The MEC generated current (on the left) and COD measurements (on the right), during operation phases 1-4.

for three consecutive cycles, with a total duration of 24 d. This required an increased NaOH addition (4.3% v / v) and an equivalent increase in initial anolyte conductivity (to 15 mS / cm). During phase 3, the generated current exhibited the same peak as that of phase 1 (approximately 1.5 mA), however the current plateau lasted for 4-5 d while it was gradually reduced to low values. Moreover, the initial COD (39.5 ± 2.4 gO_2 / L) was reduced to 5 ± 1.2 gO_2 / L for each cycle. The total phenolic content was reduced from 2.9 eq_g$_{Gallic\_Acid}$ / L initially to approximately 0.5 eq_g$_{Gallic\_Acid}$ / L over the course of each cycle, while the cumulative VFAs were 100% degraded. The average CE exhibited a rapid decrease, from 18% during phase 2, to 4% during phase 3, which is probably attributed to the hefty increase in the initial organic loading.

Regarding the organic constituents degradation, the MEC performance was improved with increasing the inlet COD concentration (from 4.1 gO_2 / L, to 5.9 gO_2 / L and to 39.5 gO_2 / L) and the anolyte conductivity (from 2 mS / cm, to 2.5 mS / cm and to 15 mS / cm). The VFAs were 100% degraded during all phases and the nature of the non-degraded COD is mostly attributed to the leftover phenols.

Effect of different applied potential on MEC performance

Finally, during phase 4, the effect of an increased applied potential was examined (1 V relative to 0.5 V for the previous phases), for four consecutive cycles, with a total duration of 36 d. The TPOMW was incorporated with no dilution, similar to phase 3. During phase 4, the generated current exhibited a current peak at 6-7 mA, and maintained a plateau during the last two cycles at approximately 4 mA for 3 d, before being gradually reduced to low values. Furthermore, the initial COD (37.5 ± 3 gO_2 / L) was reduced to 3 ± 0.6 gO_2 / L over each cycle. The total phenolic content was reduced from 2.9 eq_g$_{Gallic\_Acid}$ / L initially to approximately 0.2 eq_g$_{Gallic\_Acid}$ / L over the course of each cycle, while the cumulative VFAs were

100% degraded. The average CE increased to 10%, relative to 4% during phase 3, confirming the enhanced ability of the increased applied potential to degrade the organic constituents towards current production (Askari et al., 2022).

Moreover, an increase in the applied potential resulted in a distinct increase in the degradation of phenols (from 79% during phase 3, to 91% during phase 4), while the anodic CE also increased (from 4% during phase 3, to 10% during phase 4), which shows the correlation between the applied potential and the degradation of phenols towards smaller metabolic intermediates, as well as the ability of the electro-active bacteria to promote the performance of the MEC (Sun et al., 2020)

3.2. Bio-energy production

Table 1 shows the CH_4 production, the cathodic FE and the obtained bio-energy surplus, for all operation phases. The CH_4 production of the MEC at Open Circuit Potential (OCP) was measured to be < 0.01 mmols. During phase 1, the CH_4 production was approximately 0.08 mmols, leading to a marginal energy deficit, relative to the energy input (-0.01 kJ). However, decreasing the TPOMW dilution during phases 2 and 3, led to an increase in CH_4 production (to 0.48 mmols and 2.33 mmols, respectively), leading to an absolute energy surplus of 0.18 kJ and 1.22 kJ, respectively. Further increase in the applied potential during phase 4, resulted in increased CH_4 (2.88 mmols) production but a similar energy surplus (0.22 kJ) as that of phase 2.Regarding the cathodic FEs in phases 3 and 4, during which the TPOMW was incorporated in the MEC undiluted, these exceed 100% (Table 1). This phenomenon has been previously observed and is attributed to CH_4 and/or H_2 side production by non-electrochemical pathways (Rousseau et al., 2019). Specifically, these are mostly associated with the antagonistic growth of

Table 1. The MEC parameters during operation phases 1-4.

Operation phase	Total COD removal (%)	Total phenols removal (%)	Produced CH_4 (mmols)	CE_{anode} (%)	$FE_{cathode}$ (%)	Energy surplus (kJ)
1	74 ± 2	73 ± 1	0.08 ± 0.02	12 ± 2	25 ± 5	-0.01 ± 0.04
2	77 ± 1	76 ± 3	0.48 ± 0.1	18 ± 5	94 ± 15	0.18 ± 0.14
3	87 ± 3	79 ± 3	2.33 ± 0.2	4 ± 1	257 ± 20	1.22 ± 0.33
4	91 ± 3	91 ± 2	2.88 ± 0.1	10 ± 2	115 ± 19	0.22 ± 0.39

acetoclastic methanogens in the electrolyte medium, which compete with the electro-active bacteria on the bio-anode over substrate consumption, while contributing to the overall CH_4 production along with the hydrogenotrophic methanogens on the bio-cathode (Ceballos-Escalera et al., 2020). Despite the fact that additional CH_4 is produced, adding to the energy and thermal yields of the system, FEs > 100% are associated with MEC malfunction, since the microbial communities change, leading to an insignificant bio-electrochemical contribution towards CH_4 production (Rousseau et al., 2019). Phase 2 exhibited the optimal cathodic FE (94.5%). The fact that it is < 100% indicates that acetoclastic methanogens, which were still present in the biofilms at some concentration, made a minor contribution to the overall CH_4 production, especially considering the CH_4 production at OCP. Phase 3 employed the TPOMW under improved conditions for their competing growth, which resulted in an exacerbated source of CH_4 production ($FE_{cathode}$ 257%). An increase of the applied potential (from 0.5 V to 1 V) during phase 4, led to an increase of the anodic CE (from 4.2% to 10.3%) and to the decrease of the cathodic FE (from 257% during phase 3 to 115% during phase 4), indicating better bio-electrochemical contribution towards CH_4 production.

4. Conclusions

The present study evaluated the treatment of a two-phase olive mill waste and the degradation of its organic constituents, with simultaneous bio-electrochemical reduction of CO_2 to CH_4, using a Microbial Electrolysis Cell. Overall, the results indicated that the cell has the ability to effectively treat the strong waste (74-91% COD removal and 73-91% phenolic content degradation), under various employed conditions (dilution, applied potential). Ultimately, the optimal cell operation, in terms of efficiency, is observed in median operating conditions (initial COD of 6 gO_2 / L and total phenols of 0.7 eq_g_{Gallic_Acid} / L), when a potential of 0.5 V is applied. An increase of the applied potential (to 1 V) allows the treatment of non-diluted TPOMW (initial COD of 40 gO_2 / L and total phenols of 2.9 eq_g_{Gallic_Acid} / L) efficiently, achieving 91% COD removal and 91% phenolic content degradation along with an energy surplus of 0.22 kJ with a significant bio-electrochemical contribution.

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