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# Enhancing biomethane production with a modulated electric field in anaerobic digestion-microbial electrolysis cell systems

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## ABSTRACT

The advantages of integrating bioelectrochemical systems into anaerobic wastewater treatment reactors have been established over the last decade. In addition to enhancing biogas yield, its integration also improves biogas quality, eliminating the need for additional purification before its utilization as an energy source. While the biochemical and electrochemical processes in a hybrid AD-MEC system with a constant external electric field are well studied, the effects of a pulsed electric field remain insufficiently explored. In this study, the influence of periodic polarization using a pulsed power supply on biogas yield from distillery wastewater (vinasse and ethanol stillage) in an AD-MEC system was investigated. The experiments were performed at three different pulsed power supply frequencies with 0.8 V–1.0 Hz (1s), 0.2 Hz (5s) and 0.1 Hz (10s). The results were compared with those obtained with a constant electric field (0.8 V) and a control mode without MEC. Low-frequency pulsed power supply (0.1 and 0.2 Hz), resulted in higher biogas yield and methane content compared to both the high-frequency mode and constant electric field. For ethanol stillage, the biogas yield increased by 20–25% relative to the control, and for vinasse by 13–16% under the optimal conditions.

**Keywords:** ethanol stillage, vinasse, BES, biomethanation, AD-MEC, modulated electric field.

## INTRODUCTION

Bioelectrochemical systems (BES) offer significant potential for advancing and upgrading existing environmental technologies. Their applications include wastewater treatment from organic and inorganic pollutants, electricity generation, and the production of biohydrogen and biomethane, making them an attractive alternative to, or complement for, conventional technologies. BES are considered a key technology in the transition to sustainable bioenergy and green chemistry. In recent years, combined anaerobic digestion (AD) systems combined with a microbial electrolysis cell (MEC) have attracted particular attention. Microbial electrolysis cells integrated with anaerobic digestion (AD-MEC) represent a promising technology for the utilization of organic waste with increased biomethane yield (Negi et al., 2025). In hybrid AD–MEC systems, electrons released during the anaerobic digestion of the organic substrate are captured by exoelectrogenic microorganisms and transferred to the anode. From there, they are directed through the external circuit to the cathode, where they serve as electron donors for methanogenic archaea, stimulating bioelectrochemical methanation (Lee et al., 2022). In this way, the classical metabolic pathways of anaerobic digestion are combined with controlled electrochemical stimulation, which increases the efficiency of bioconversions (Ao et al., 2024). In the anode zone, organic matter is oxidized by electroactive bacteria that release electrons and protons (Garbini et al., 2023). The electrons are directed to the cathode, where an applied external voltage (exceeding the thermodynamic threshold of ~0.2

V for methanogenesis) facilitates the reduction of $CO_2$ to $CH_4$ by electrotrophic methanogens or catalyzed reactions involving hydrogen intermediates (Cheng et al., 2009).

A key mechanism for increasing efficiency in AD-MEC is direct intercellular electron transfer (DIET). It reduces the dependence on traditional mediators ($H_2$ and formate) and provides a faster and energetically favorable electron flow between syntrophic bacteria and methanogens. The application of an electric field in MEC not only stimulates DIET but also supports the stability of microbial consortia, including when loaded with difficult to degrade organic substrates (Barua and Dhar, 2017).

Historically, the first attempts to integrate MEC into anaerobic reactors date back to the early 2000s (Logan et al., 2008), when it was shown that the application of a low external voltage could increase methane production by over 30–40% compared to standard anaerobic digestion. Since then, AD-MEC has been developing as an actively researched approach, aimed both at improving the energy balance of anaerobic systems and at treating more complex waste streams – distillery wastewater, food waste, sewage sludge, etc. Thus, AD-MEC systems offer many advantages: increased methane yield, faster decomposition of organic matter, improved biogas quality (with higher $CH_4$ content and lower concentrations of $CO_2$ and $H_2S$), as well as the potential for energy-efficient treatment of waste streams (Wang et al., 2022).

One of the key factors significantly influencing the efficiency of bioelectrochemical systems is the formation of polarization diffusion barriers in the anode and cathode regions in the presence of a constant external electric field. These barriers are believed to impede the transport of current carriers both to the electrode surface and through the membranes (Li and Anand, 2016). The accumulation of ions near the electrodes and membranes leads to the formation of an electric double layer (EDL) and concentration polarization barriers. They limit the transport of current carriers and reduce the efficiency of electrochemical processes. In recent years, pulsed electric field (PEF) has been applied as an effective method to overcome these limitations. Experimental results show that the use of PEF in electrodialysis systems leads to a significant reduction in concentration polarization and improved mass transfer (Rybalkina et al., 2022). This phenomenon is explained by the periodic "destruction" of depleted ion layers and stimulation of local electroconvection.

Mathematical models further show that the application of a pulsed voltage of varying amplitude alters the dynamics of the ionic profiles and reduces the stability of the stacked layers, which in turn reduces the electrical resistance of the membrane (Gorobchenko et al., 2021). Physicochemical studies of EDL confirm that pulsed regimes lead to different dynamics of formation and relaxation of the double layer compared to the constant field. This indicates that PEF can control both the thickness and structure of the EDL (Xu et al., 2018).

Until now, periodic polarization in BES, and in particular in AD-MEC, has been poorly studied as a factor for optimizing bioelectrochemical methanation in this type of hybrid system. In several studies (Szwarc and Szwarc, 2021; Kovačić et al., 2021; Straessner et al., 2023), a pulsed electric field has been used to pre-treat organic substrates before the anaerobic digestion process, achieving a higher degree of substrate utilization and increasing their biogas potential. In another study (Hussain et al., 2018), the application of periodic power interruption with a frequency of 0.1–0.5 Hz and a duty cycle of 90–95% to a microbial electrolysis cell (MEC) leads to a gradual increase in COD removal efficiency from 80% to 90% and a decrease in the internal resistance of the system.

The main objective of this study is to evaluate the influence of periodic polarization by a low-frequency pulsed power supply on AD-MEC. It is expected that this approach will contribute to overcoming the polarization diffusion barriers in the cathodic (resp. anodic) zone, to increase the current density and to reduce the internal resistance of the system. In this way, it is expected to achieve higher efficiency of AD-MEC, expressed in increased biogas yield and more complete absorption of organic substrates.

## MATERIALS AND METHODS

### Design of the laboratory installation and process operation

A laboratory setup (Figure 1) was used to implement the experiment, including – a vessel with a feed organic substrate (1), a dosing peristaltic pump (2), an anaerobic bioreactor (UASB) for biomethanation with a working volume of 3.0 $dm^3$ (3), a recirculation pump (6), a microbial

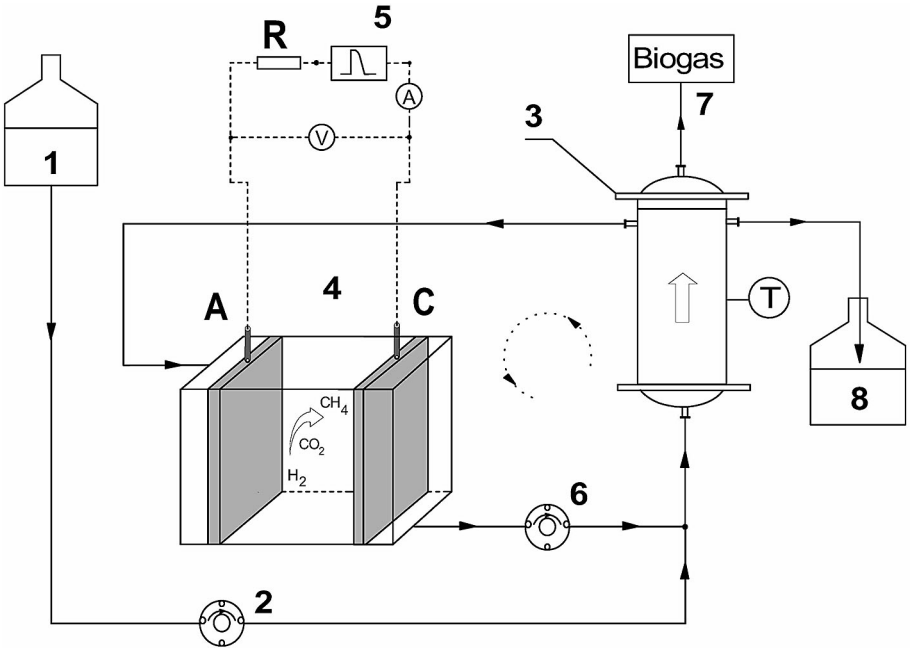


**Figure 1.** Configuration of the laboratory-scale AD-MEC reactor. 1 – feed substrate, 2 – dosing peristaltic pump, 3 – UASB-reactor, 4 – MEC, 5 – loading circuit of MEC with pulse generator, 6 –recirculation loop between UASB and volume of MEC, 7 – biogas, 8 – effluent substrate

electrolysis cell (MEC) with a volume of 150 $cm^3$ (4), a load circuit of the MEC with a controllable pulse generator and external resistance – R = 10 Ω (5), an outlet biogas (7) and an outlet for spent substrate (8). During the experiments, the laboratory installation operated in continuous mode, with the flow rate of the feed pump (1) set to provide a contact time of 10 days.

Two types of waste organic substrates were used for the experiments – vinasse (from wine brandy production) and ethanol stillage (from an ethanol plant obtained by hydrolysis of wheat using a sulfuric acid solution). These substrates are characterized by a high chemical oxygen demand (COD) between 25 and 75 $gO_2/L$, low pH (3.5–4.0) and a balanced content of macro- and microelements. These substrates also contain easily degradable organic matter, which makes them a potential substrate for methanation with the generation of methane-rich biogas (de Carvalho et al., 2023; Zielińska et al., 2012). Given the high initial COD values, vinasse and ethanol stillage were prediluted in a ratio of 1:1 with distilled water, and the pH was adjusted to 7.5 using a 4N NaOH solution. Thus, the initial COD values for the used feed solutions (1) of vinasse and stillage were 30.1 and 42.9 g $O_2/L$, respectively. A complete characterization of the organic composition of the organic substrates used in this study was done in a previous study (Velichkova et al., 2025). During the experiments, the solutions with organic substrates were stored at a temperature of 4 °C in a refrigerator. The inlet waters entered the laboratory installation with a contact time for the AD-BES system of 10 days, which was established in a previous study (Velichkona et al., 2022), as optimal for the organic substrates used. For the microbial electrolysis cell (MEC), a two-chamber "sandwich" type structure made of Plexiglas was used, using graphite plates with dimensions of 6 × 100 × 100 mm. The working volume of the chamber in which the two electrodes are located is 150 ml.

Two groups of studies were conducted, respectively for the two organic wastewaters – vinasse and ethanol stillage. For each of the substrates, the following options were tested – control with only AD and AD-MEC at a constant voltage with an amplitude of Vp = 0.8 V, as well as 3 options with pulsed power supply at three different frequencies – 1.0 Hz (1s), 0.2 Hz (5s) and 0.1 Hz (10s), with all pulsed impacts having a voltage amplitude of Vp = 0.8 V and duty cycles d = 90%. The choice of the frequency range of the pulsed impacts (0.1–1.0 Hz) and the duty cycle (90%) was selected based on (Hussain et al., 2018).

The methanogenic reactor was inoculated with activated sludge sourced from an up-flow anaerobic sludge blanket (UASB) reactor treating wastewater from a bioethanol production facility. The culture consisted of granulated biomass in

the form of spherical flocs with diameters of 2–3 mm. These pellets exhibited good mechanical stability (Velichkova et al., 2017).

## Pulse generator configuration

The MEC driver circuit is implemented using a low-noise, low-frequency pulse voltage source that provides control over the voltage amplitude and pulse parameters, such as duration and repetition period. For this purpose, a dedicated pulse generator circuit (Figure 2) was designed, employing a low-noise, amplitude-controlled voltage source to supply the MEC. The programmable LabJack T7-Pro device served as both the pulse generator and the data acquisition (DAQ) module, offering a 14-channel 24-bit ADC, 23 digital inputs/outputs, two 12-bit analog outputs, and configurable counters.

The behavior of the MEC cell is analogous to that of an organic battery, with a variable internal resistance $R_i$. Moreover, the output impedance of DAC0 is relatively high (~180 Ω), which causes the applied voltage to be influenced by the MEC load. To address this, the circuit incorporates an analog multiplexer (U1) and an operational amplifier (U2) configured as a voltage follower. The output impedance of U2 is negligibly small, ensuring both the stability of the applied voltage and the insensitivity of the circuit to variations in the internal resistance of the MEC across a wide operating range.

In Figure 2, GND represents the zero potential, DAC0 is the programmable analog output, FIO0 is the output of the pulse generator, and AIN0 and AIN1 are analog inputs. $R_o$ is a low-impedance shunt resistor used to measure the circuit current. The multiplexer U1 routes the programmed voltage (~0.8 V) to the voltage follower under the control of pulses generated by FIO0. With the set-up shown, a concise mathematical analysis of the energy performance can be carried out. The area under the discharge curve of the produced pulse allows for determining the energy ($E_p$) released during the pulse- Equation 1 (Santoro et al., 2016):

$$E_p = I_p \int_{t1}^{t2} V_p dt = P.(t_2 - t_1) = P.t_p \quad (1)$$

where: $I_p$ is the applied current pulse, $V_p$ is the pulse voltage, $t_p$=$t_2$-$t_1$ is the pulse duration, and $P$ is the power.

The current through the circuit when a pulse is applied is Equation 2:

$$I(t) = \frac{V_p}{R_o + R_i} \quad (2)$$

where: $R_o$ is the shunt resistor, across which the voltage drop is measured to obtain the current, and $R_i$ is the internal resistance of the MEC, representing the sum of internal ohmic, activation, and concentration resistances within the microbial electrolysis cell.

The power delivered to the load is calculated as Equation 3:

$$P = I_p^2.R_o.\frac{V_p^2.R_o}{(R_o + R_i)^2} \quad (3)$$

For a period $T$ of 10s, and some duty cycle $\alpha$ (0.8 and 0.2), the average power is Equation 4:

$$P_{avg} = P.\alpha = I_p^2.R_o.\frac{V_p^2.R_o.\alpha}{(R_o + R_i)^2} \quad (4)$$

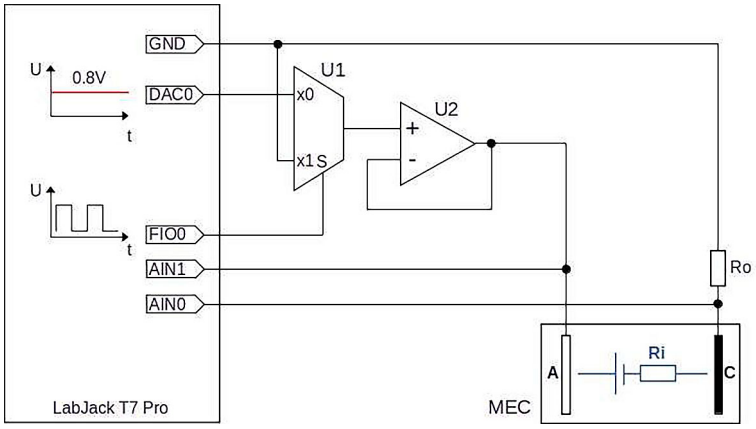


**Figure 2.** Pulse generator configuration for MEC power supply

Numerical estimates show that for $R_i << R_o$, $P \approx (V_p)^2/10 = 0.064$ W. With $\alpha$=0.8, $P_{avg} \approx 0.0512$ W, and with $\alpha = 0.2$, $P_{avg} \approx 0.0128$ W. For $R_i = 10\Omega$, P = 0.016 W. With $\alpha = 0.8$, $P_{avg} \approx 0.0128$ W, and with $\alpha = 0.2$, $P_{avg} \approx 0.0032$ W which is a fourfold reduction of the average power. These results highlight the strong influence of the internal resistance on the actual energy input. Therefore, real-time measurement of $R_i$ via the integrated shunt resistor and ADC inputs is essential to correctly evaluate the energy transfer. In Rahimi and Eicker (2023) and Santoro et al. (2016) a detailed analysis of the electrochemical equations were made. We only need to mention here that the above setup allows for the calculation of the required external voltage $V_p$ (Rahimi and Eicker, 2023)- Equation 5:

$$V_p = E_{ep} - \eta_{ohm} - \eta_c - \eta_{act} \quad (5)$$

where: $E_{ep}$ is the electrode potential, $\eta_{ohm}$ the ohmic, $\eta_c$ concentration, and $\eta_{act}$ activation losses at the anode.

The schematic diagram implementing the buffering circuit is shown in Figure 3a, and the experimental setup is shown in Figure 3b.

The proposed pulse generator design provides a versatile platform not only for controlling energy input but also for enabling more advanced experiments. Equation 4 allows the identification of minimal $P_{avg}$ that still reduces polarization barriers and enhances methane yield. Applying pulse spectroscopy and varying frequency and duty cycle, would allow for the measurement of the relaxation times of the electrical double layer, providing insights into how mass transfer may be limited. Frequency scanning (0.05–1 Hz) combined with duty-cycle variation enables minimization of power consumption while maximizing methane production. The generator functionality can be further extended as it allows for the implementation of sinusoidal or triangular signals for frequency analysis; and feedback control of pulse parameters based on measured current or internal resistance.

## Analytical methods and electrochemical analysis

In the laboratory setup, measurements were taken for pH, redox potential (Eh), and electrical conductivity (EC) Milwaukee MW180 (Hungary). The organic load was determined via chemical oxygen demand (COD) analysis using a HANNA Instruments kit. Organic acids were quantified by high-performance liquid chromatography (HPLC) employing an Acclaim Organic Acid column coupled with a UV-Vis detector. The mobile phase consisted of 0.1 M $Na_2SO_4$, applied at a flow rate of 0.6 mL/min with an injection volume of 5 µL. Gas volume was recorded using a MilliGascounter 'Ritter MGC-1', while the concentrations of $CO_2$ and $CH_4$ in the biogas were measured with a portable gas analyzer 'Draeger X-am 7000'.

The electrical parameters of the MEC were monitored using a digital multimeter (Fluke 115). The power density (P), normalized to the geometric surface area of the anode, was calculated according to the equation $P = U^2/(R_T.A)$, where A ($m^2$) represents the anode surface area, $R_T$ ($\Omega$) is the external load resistance, and U(V) is the measured MEC voltage. The current in the MEC was quantified by measuring the voltage drop across the external load resistance ($R_T$ = 10 **Ω**) and subsequently calculated using Ohm's law.

Coulombic efficiency (CE) was evaluated based on the difference in COD (ΔCOD) between the influent and effluent, together with the average current recorded during the experiment. The CE value was calculated using the following formula:

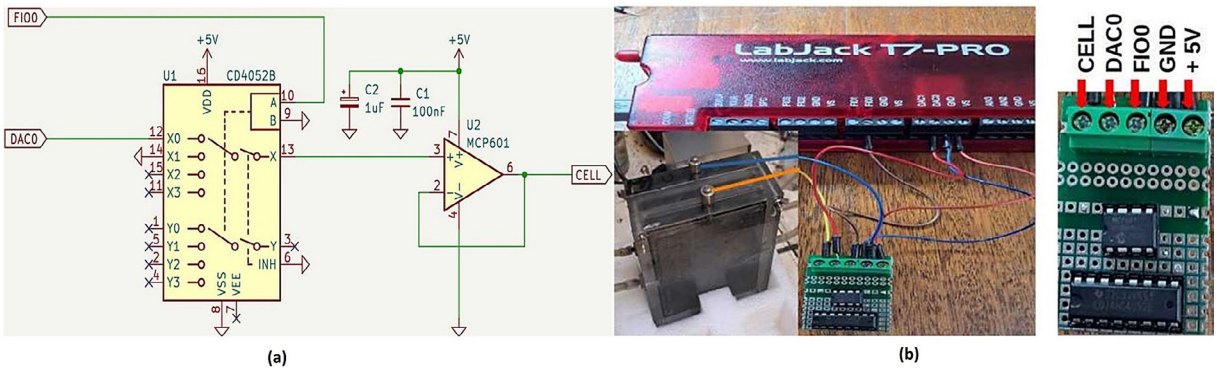


**Figure 3.** (a) Schematic diagram of the decoupling buffer of the pulse generator, (b) experimental setup

$$CE = \frac{M.I.t}{F.b.V.\Delta COD.} x100\% \quad (6)$$

where: $M$ = 32 – the molar mass of $O_2$, $t$(s) – substrate residence time in AD-MES, $I$ – average current value during the experiment (A), $F$ (Faraday constant) = 96845 C/mol, $b$ = 4 – number of electrons needed to oxidize 1 mol $O_2$, $\Delta COD$ – difference between the initial and final value of COD ($gO_2$/L), $V$ – working volume of AD-MES (L).

Similarly, the following formula was used to calculate methane recovery efficiency (MRE):

$$MRE = \frac{8.M.n_{CH_4}}{b.V.\Delta COD.} x100\% \quad (7)$$

where: 8 – the number of electrons needed for the reduction of 1 mol $CO_2$ to $CH_4$, $M$ = 16 – the molar mass of $CH_4$, $b$ = 4 – number of electrons needed to oxidize 1 mol $O_2$, $n_{CH4}$ — moles of methane produced (or equivalent volume converted to moles), $\Delta COD$ – difference between the initial and final value of $COD$ ($gO_2$/L), $V$ – working volume of AD-MES (L).

It should be noted that

$$n_{CH_4} = \frac{V_{CH_4}}{22.4} \quad (8)$$

where: 1 mole of an ideal gas occupies 22.4 L/mol at standard temperature and pressure (STP), and $V_{CH4}$ - is the generated, cumulative volume of $CH_4$ (L) over the time of the experiment.

The total electrical energy consumed $W_E$, total energy revenue $W_{CH4}$ and energy efficiency $\eta_E$ are determined according to Zhao et al., (2016). Thus, the total consumed electrical energy $W_E$ (J) at the applied external voltage E in MEC-AD systems is calculated according to Equation 9:

$$W_E = I.E.\Delta t - I^2.R.\Delta t \quad (9)$$

where: $I$ (A) is the average value of current generation, $E$ (V) is the 0.8 V potential difference applied between anode and cathode, $\Delta t$ (s) is the voltage applied time, and $R$ is the 10 Ω resistance on the circuit.

Total energy revenue $W_{CH4}$ [J] was calculated by Equation 10:

$$W_{CH4} = \frac{\Delta H_s (V_{AD-MEC} - V_{Control})}{V_{mv}} \quad (10)$$

where: $\Delta H_s$ is the calorific value of $CH_4$ combustion (890.31 × 103 J/mol), $V_{MEC-AD}$ (L) and $V_{Control}$ (L) is the cumulative $CH_4$ yield of the different variants during the whole experiment and $V_{mv}$ is the molar volume of gas at 25 °C and 1 atm (24.465 L/mol).

Energy efficiency $\eta_E$ (%) is defined by the below Equation 11:

$$\eta_E = \frac{W_{CH4}}{W_E}.100\% \quad (11)$$

## RESULTS AND DISCUSSIONS

To enhance the electrochemical performance of the microbial electrolysis cell (MEC) under a periodically modulated electric field, the system was subjected to square-wave voltage excitation at different frequencies ranging from 0.1 to 1 Hz. This task was accomplished with the aid of the low-frequency pulse generator discussed above. The modulation was intended to stimulate microbial activity, improve charge transfer between the biofilm and the electrode, and promote faster recovery of the electrochemical interface between cycles. In Figure 4 the temporal evolution of the measured voltage (Vmec) and current for various modulation frequencies is presented. At 1.0 Hz, the waveform exhibits sharp periodic voltage drops with incomplete recovery between cycles, suggesting that the time constant of the electrochemical interface is comparable to or longer than the modulation period. As the modulation frequency decreases to 0.2 Hz and further to 0.1 Hz, the voltage transitions become slower and more pronounced, with the system reaching quasi-steady states during each half-cycle. This frequency-dependent response indicates a capacitive character typical of microbial electrochemical systems, where both the double-layer charging and microbial electron transfer contribute to the observed current.

The voltage in the MEC is governed by the external power supply and the internal resistance of the electrochemical system, which includes ohmic, activation, and concentration losses. The periodic voltage drops observed in Figure 4 likely reflect transient increases in internal resistance or localized bioelectrochemical limitations. Possible

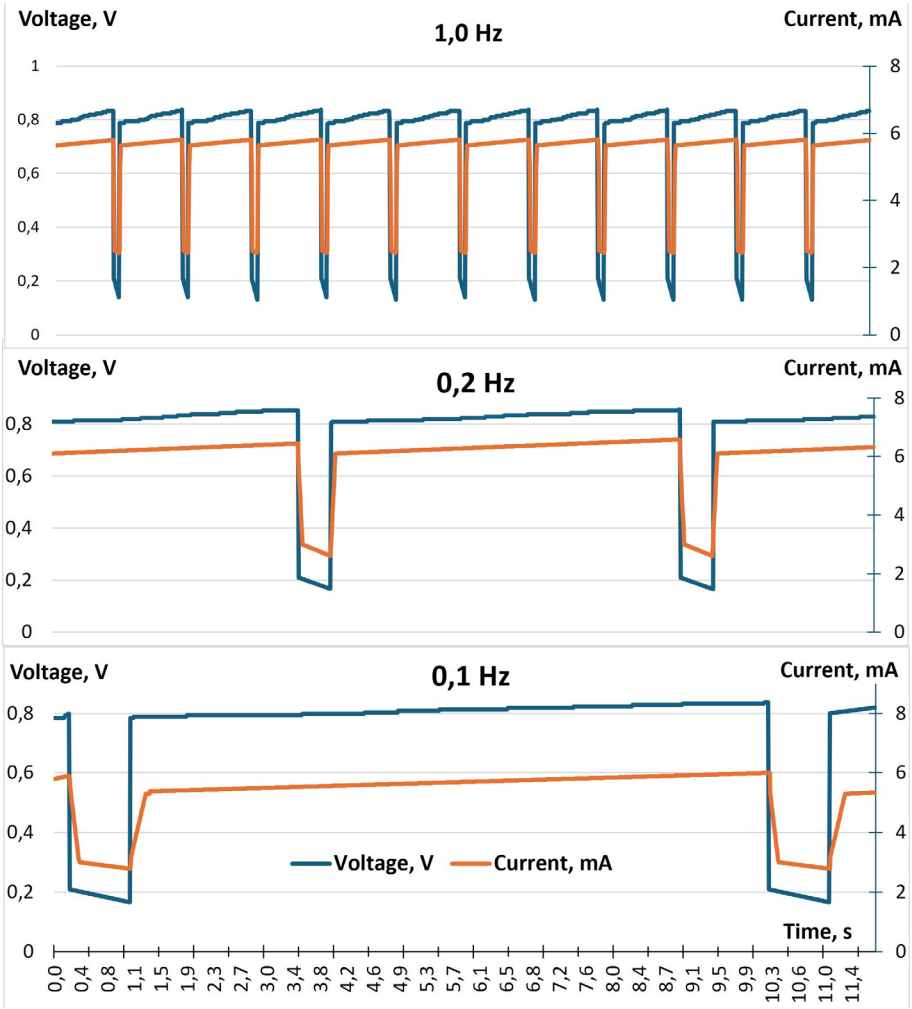


**Figure 4.** Voltage and current dynamics under a modulated electric field

contributing mechanisms are the temporary degradation or detachment of the biofilm, altering the effective conductive pathway, the local substrate depletion near the bioanode, reducing microbial oxidation rates, and the gas accumulation (e.g., $H_2$) at the cathode, which increases the overpotential and reduces the effective potential difference across the electrodes. These factors lead to a decrease in the available voltage for the electrochemical reactions, producing the observed periodic declines and recoveries.

The non-linear current response behavior is typical of bioelectrochemical systems. In a MEC, current generation arises from the microbial oxidation of organic substrates at the anode and the hydrogen evolution reaction at the cathode. The sharp current spikes correspond to periods of high microbial activity and efficient electron transfer. Subsequent drops of the current indicate transient limitations within the system, which may be attributed to the rapid substrate exhaustion near the biofilm interface, the accumulation of inhibitory products such as hydrogen gas at the cathode, and the bioanode passivation or electrode fouling, which temporarily hinder electron transfer. After a short relaxation phase, diffusion of substrate and removal of inhibitory products restore system balance, allowing the current to rise again in the next modulation cycle.

The kinetics of biogas production in the studied variants are presented in Figure 5. A clear increase in the amount of biogas produced is observed in all variants with the integration of AD-MEC compared to classical anaerobic digestion (AD-Control).

When comparing the two substrates used, it was found that the ethanol stillage provided a higher overall biogas yield compared to the vinasse, regardless of the electrochemical treatment mode. This is mainly due to the higher initial concentration of organic substances (COD = 42.9 g $O_2$/L for sludge, compared to 30.1 g $O_2$/L for vinasse).

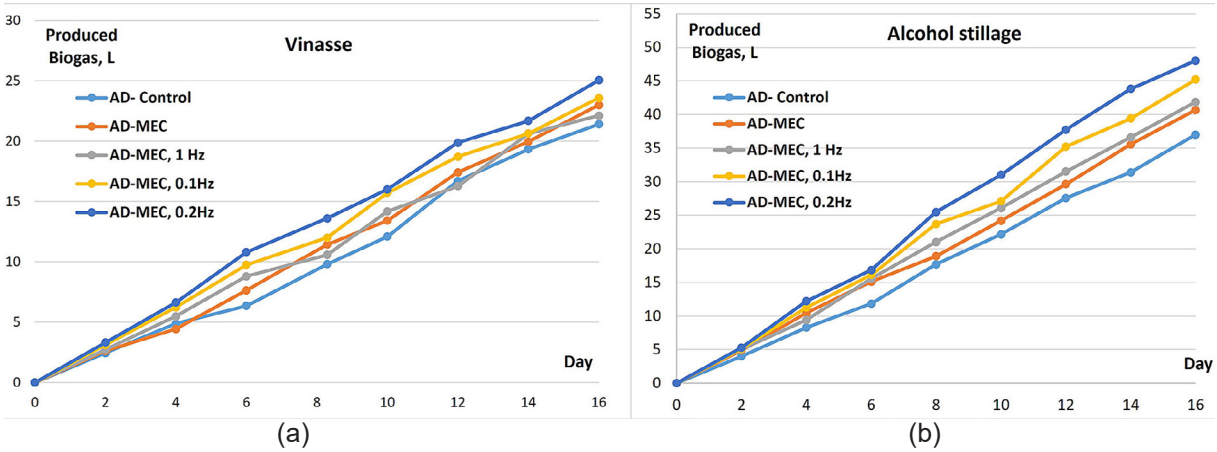


**Figure 5.** Kinetics of biogas production from vinasse (a) and ethanol stillage (b) in different variants of modulated electric field in AD-MEC

Among the studied AD-MEC modes, the highest efficiency was recorded at pulsed polarization with a frequency of 0.2 Hz, both for the stillage and the vinasse. In the stillage, the effect of electrochemical support of methanogenesis is more pronounced, especially at low frequencies (0.1 and 0.2 Hz), where a significant increase in biogas yield is observed compared to the control system. At a higher frequency (1.0 Hz), the produced biogas amounts are close to those at a constant electric field (AD-MEC, 0.8 V), which suggests that at this frequency the effect of periodic polarization is partially limited.

At the end of the 16-day experimental period (Figure 5), an increase in the accumulated biogas yield was observed for all variants with AD-MEC compared to the control. The most significant increases were observed for the stillage – by 25.1% and 20.4%, respectively, at frequencies of 0.2 Hz and 0.1 Hz. For the vinasse, the increases were more moderate – by 15.9% and 12.5%, respectively, at the same frequencies. Figure 6 presents the methane content in the produced biogas, and Table 1 shows the daily biomethane yield. It was found that in vinasse, the methane content increased by 7.9% in the AD-MEC mode (0.8 V, 0.2 Hz, duty cycle 90%), with the daily methane yield being 26.3% higher than the control. In stillage, the increase in methane content was 7.6%, and the daily yield increased by 39.6%.

**Table 1.** Daily biomethane yield in different operating modes

| Mode | Vinasse, L/day | Ethanol stillage, L/day |
|---|---|---|
| AD- Control | 0.84 | 1.53 |
| AD-MEC, 0.8 V | 0.95 | 1.73 |
| AD-MEC, 0.8 V, 1.0 Hz | 0.94 | 1.78 |
| AD-MEC, 0.8 V, 0.1 Hz | 1.03 | 1.95 |
| AD-MEC, 0.8 V, 0.2 Hz | 1.07 | 2.13 |

The constant voltage (AD-MEC, 0.8 V) and higher pulse frequency of 1.0 Hz variants showed similar values in terms of methane content and daily yield, confirming that the optimal effect occurs at lower pulse polarization frequencies (0.1 and 0.2 Hz). These results demonstrate that periodic electrochemical impact can activate the methanogenic community and improve the kinetics of biogas generation, especially with more concentrated organic substrates such as ethanol stillage.

The analysis of the technological parameters for the two substrates (vinasse and ethanol stillage) presented in Table 2 reveals a pronounced positive effect of the electric field on methanogenesis, especially at low frequency pulsed supply (0.1–0.2 Hz). This leads to a significant increase in both biogas yield and energy efficiency ($\eta_E$) compared to the control mode AD-control.

The MRE values under pulsed energization are always higher (for both substrates) compared to constant energization, suggesting better electron flow targeting towards methanogenic metabolic pathways. This may be due to improved mass transfer and overcoming diffusion barriers, as well as reduced cathodic overvoltage during switching, which facilitates carrier transfer and stimulates methanogenic bacteria. Similar trends were observed by Zakaria et al. (2022), where intermittent energization stimulates DIET and increases methanogenesis. By Hussain et al. (2018), periodic on/off cycles in MEC lead to increased current and higher COD removal

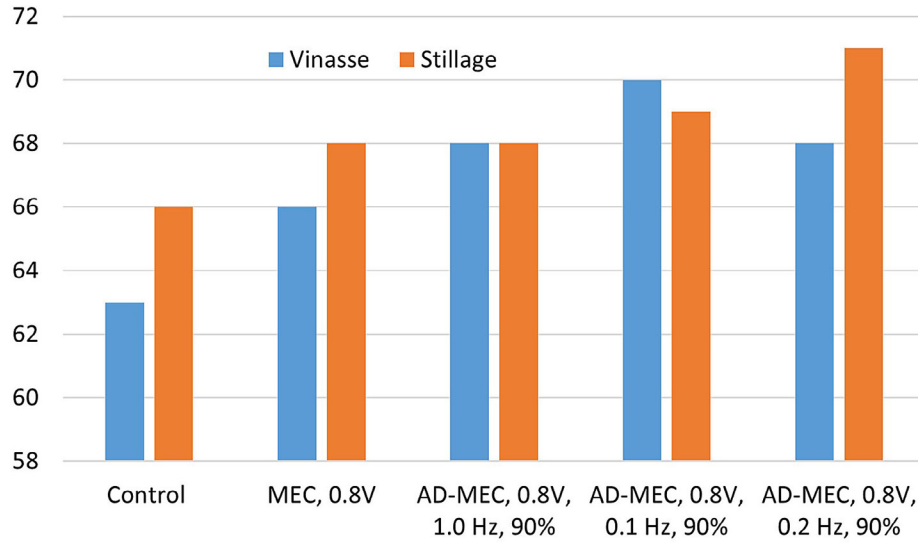


**Figure 6.** Methane content in the produced biogas from different operating modes of the installation

compared to constant energization. The low values of the coulombic efficiency (CE < 1%) in the vinasse and ethanol stillage in all studied variants can be explained by the high organic load and the presence of sulfates, which serve as a competitive electron acceptor for the sulfate-reducing bacteria. This competition redirects part of the electron flow from the anode to intracellular or diffuse pathways (electron sinks), which reduces the part passing through the external electrical circuit. A similar phenomenon was discussed by Yuan et al. (2020), where the competition between sulfate-reducing bacteria and methanogens was identified as a factor limiting CE in integrated MEC-AD systems, while on the other hand, in the same publication, it was shown that the use of combined AD-MEC systems reduces the inhibition by sulfides, improving both the biomethanation process and energy recovery.

Overall, the obtained CE and MRE values are relatively low compared to other similar studies (Vrieze et al., 2018). This may be due to various reasons – high internal resistance of the medium, the use of complex substrates such as vinasse and stillage, insufficient electrochemical activity of the biofilms formed on the electrodes, the presence of competing metabolic pathways such as microbial sulfate reduction and nitrate reduction in the presence of nitrates and sulfates in the medium, the use of ordinary graphite electrodes with a small specific surface area close to the geometric one, etc.

In addition, higher average current values are observed in pulsed modes compared to the constant current mode (AD-MEC, 0.8 V), which is probably due to the periodic discharge of the electrochemical double layer and the easier overcoming of the diffusion barriers in the anodic and cathodic zones. As a result, the mass transfer of ions and metabolites is facilitated and the efficiency of electron transfer between the electroactive bacteria and the electrodes is improved. A similar effect has been reported in experimental studies of pulsed MEC/AD-MEC systems (Hussain et al., 2018), where an increase in the average current and biogas yield is observed at an optimal frequency of about 0.5–1.0 Hz.

The total electrical energy consumed ($W_E$) varies within the range of 2.5–4.0 kJ, while the energy equivalent of the methane produced ($W_{CH_4}$) reaches 15.7 kJ (stillage), which indicates that the input electrical energy is repeatedly recovered through biogas production. The energy efficiency ($\eta_E$) exceeds 100 % for all AD-MEC variants and reaches a maximum of 522 % for vinasse (0.8 V, 0.1 Hz). This indicates that the electrochemical effect not only converts electrical energy into chemical energy but also stimulates the biological processes of methanogenesis through improved electron transfer and activation of syntrophic microbial interactions.

The lower $\eta_E$ values in vinasse are likely due to the more complex and inhibitory nature of its organic matrix, containing phenolic and aromatic compounds that suppress the metabolic activity of acetogenic and methanogenic microorganisms Silva et al. (2021).

The increased $\eta_E$ values under low-frequency pulsed power supply can be further explained by the activation of syntrophic microbial interactions

**Table 2.** COD removal rate, Average current ($I_{avg}$), Coulombic efficiency (CE), $W_E$, $W_{CH4}$, $\eta_E$ and methane recovery efficiency (MRE) under different operating modes for vinasse and ethanol stillage

| Vinasse | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mode | COD, % | ΔCOD, g/l | $I_{avg}$, mA | CE, % | MRE, % | $W_E$, J | $W_{CH4}$, J | $\eta_E$, % |
| AD- Control | 93.0 | 28.0 | - | - | 14.6 | - | - | |
| AD-MEC, 0.8 V | 94.7 | 28.5 | 3.81 | 0.38 | 16.1 | 2508 | 3 433 | 137 |
| AD-MEC, 0.8 V, 1.0 Hz, 90% | 95.2 | 28.7 | 4.12 | 0.41 | 17.3 | 2701 | 6 110 | 226 |
| AD-MEC, 0.8 V, 0.1 Hz, 90% | 96.0 | 28.9 | 4.32 | 0.43 | 21.4 | 2825 | 14 746 | 522 |
| AD-MEC, 0.8 V, 0.2 Hz, 90% | 96.7 | 29.1 | 4.44 | 0.44 | 19.0 | 2899 | 10 117 | 349 |
| Ethanol stillage | | | | | | | | |
| Mode | COD, % | ΔCOD, g/l | $I_{avg}$, mA | CE, % | MRE, % | $W_E$, J | $W_{CH4}$, J | $\eta_E$, % |
| AD- Control | 95.8 | 41.1 | - | - | 23.88 | - | - | |
| AD-MEC, 0.8 V | 96.9 | 41.6 | 5.14 | 0.35 | 25.51 | 3325 | 5622 | 169 |
| AD-MEC, 0.8 V, 1.0 Hz, 90% | 97.2 | 41.7 | 5.80 | 0.39 | 25.90 | 3718 | 6950 | 187 |
| AD-MEC, 0.8 V, 0.1 Hz, 90% | 98.1 | 42.1 | 5.90 | 0.40 | 24.85 | 3777 | 4565 | 121 |
| AD-MEC, 0.8 V, 0.2 Hz, 90% | 98.4 | 42.2 | 6.30 | 0.43 | 28.53 | 4012 | 15660 | 390 |

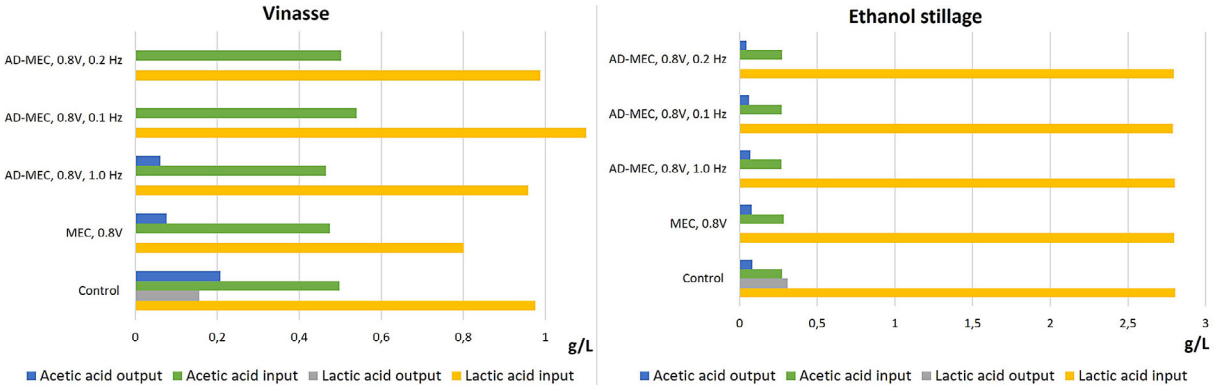


**Figure 7.** Lactic and acetic acid content at the inlet and outlet of the installation under different operating modes

and direct intercellular electron transfer (DIET) facilitated by electroactive species such as Geobacter and Desulfovibrio (Chen et al., 2022), typically present in methanogenic consortia.

Figure 7 shows the reduction of lactic and acetic acid in both substrates in the 5 studied modes. It is clearly seen that lactic acid is completely degraded in the MEC modes compared to the control, where 0.31 g/L remains in the case of the ethanol stillage and 0.16 g/L in the vinasse, compared to the initial concentration of 2.8 g/L and 1.0 g/L, respectively. The results for acetic acid show that in the case of vinasse, its degradation is incomplete in the control, the MEC 0.8 V mode and in the pulsed mode with 1.0 Hz, while in the low-frequency regime (0.1 and 0.2 Hz) it is complete. For the stillage, improved degradation is observed in the direction of control < MEC 0.8 V < MEC 0.8V 1.0 Hz < MEC 0.8 V 0.1 Hz < MEC 0.8 V 0.2 Hz. The results correlate with those for the methane content in biogas (Figure 6), with the increase most likely being due to the activation of electroactive bacteria upon integration of the MEC into the system. According to Sravan et al. (2024), there is a strong correlation between bacterial electrometabolic activity and methane formation, with 0.8V improving the efficiency of methane production from acetate, hydrogen and $CO_2$. In a closed circuit, microbial growth, metabolic activity and the rate of methane production are accelerated compared to a closed circuit, where electron transfer relies solely on microbial activity, which is strongly influenced by the conditions in the reactor.

## CONCLUSIONS

The application of bioelectrochemical systems in the analytical degradation of wastewater with a complex composition is an innovative

approach to increase the production of biomethane and the degradation of organic content. The obtained results show that periodic polarization supports electron transfer and overcomes diffusion limitations in the anode zone. In addition, the energy efficiency exceeds 100% in all variants of the MEC integrated system, with the best results observed in the low-frequency power supply. In the case of vinasse, the methane content increases by 7.9% at 0.2 Hz, and in ethanol stillage by 7.6% vs. control modes.

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