

INTRODUCCION

Anaerobic digestion (AD) is a well-established bioprocess that converts organic waste into biogas and digestate through anaerobic fermentation. The biogas, primarily composed of methane (CH₄) and carbon dioxide (CO₂), can be used for heating, electricity generation or as biofuel. The remaining semi-solid byproduct, known as digestate, is nutrient-rich and has significant potential for use in composting and soil improvement. Bioelectrochemical systems (BES) use electroactive microorganisms that interact with electrodes as electron acceptors, where applied voltage can modulate their metabolism and accelerate anaerobic digestion kinetics⁽¹⁾. **The main objective of the Waste4Soil project is to demonstrate and optimize a pre-industrial AD-BES pilot plant.** The system aims to improve biogas production and waste valorization by integrating a BES module and testing co-digestion of regional agri-food residues.

Key objectives include:

- 1) Testing **different co-digestion strategies** using local agro-food industry wastes.
- 2) **Optimizing the BES applied voltage** to improve system performance and methane yield.

MATERIAL AND METHODS



Fig 1. AD-BES pilot plant



Fig 2. BES reactor

Operation:

- pH 7,7 and T 35°C
- HRT 35 days
- Inoculum: AD sludge from local wastewater treatment plant
- Constant mixing by recirculation

A pilot AD-BES system was constructed (Fig 1), consisting of a 550 L jacketed, airtight stainless-steel tank (AD), coupled with a 30 L single-chamber BES module (Fig 2) integrated into the recirculation loop. The BES module included four anodes and four cathodes (0,3 m²), all made of carbon brushes, connected to a power supply. The AD-BES reactor featured a gas outlet connected to a gas counter, with the produced gas subsequently stored in a gas bag for analysis. The feed was introduced twice a week, maintaining a hydraulic retention time (HRT) of 35 days. The digestate was automatically discharged into an adjacent settling tank.

4 substrates were selected based on regional production and seasonality in Catalonia (Spain). First, two annual residues, beer bagasse (BB) and pig manure (PM); second two seasonal residues, wine vinasse (WV) and olive mill (OM). A base co-digestion of **66% PM and 33% BB (S1)** was selected to maintain a good C/N ratio and used to initiate the reactor and stabilized it. Then two different mixtures were tested to assess the best seasonal residue:

S2 = 50% PM + 33% BB + 17% WV



S3 = 50% PM + 33% BB + 17% OM

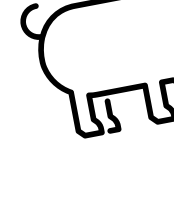
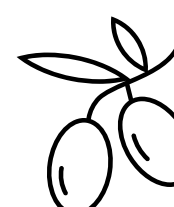


Table 1. Physicochemical characterization of co-digestion mixtures

	pH	Conductivity (mS/cm)	COD total (g/L)	N-NH4 (mg N/L)	TS (g/L)	VS (g/L)	FOS/TAC	OLR g COD/day/L
S1	7,60	10,06	14,43	830	19,56	13,9	0,74	0,41
S2	7,6	8,81	8,11	655	12,03	8,37	0,34	0,23
S3	7,54	6,26	10,63	416	13,14	9,21	0,66	0,30

RESULTS

1) CO-DIGESTION OPTIMIZATION

- S2 increased CH₄ yield (0.23 L/kg VS) but led to low COD removal (7%), suggesting possible AD inhibition due to presence of recalcitrant compounds (Table 2).
- S3 improved both CH₄ production (0.29 L/kg VS) and COD removal efficiency (43%) compared to the base substrate S1.

Table 2. Results of the AD-BES pilot in each feedstock phase

	CH ₄ rate (L/ kg SV)	CH ₄ (%)	COD removal (%)	VS removal (%)	Current density (A/m ²)
S1	0,16	67	41	33	0,1
S2	0,23	64	7	16	0,14
S3	0,29	67	43	38	0,16

2) VOLTAGE OPTIMIZATION

Once the S3 mixture was identified as the optimal co-digestion substrate, voltage optimization was carried out. The initial applied voltage was a low value, 0.3 V, to ensure biofilm development, resulting in an average current of 0,06 A/m² and electro-fermentation coefficient of 0,18%. However, increasing the voltage to 0.6 V did not improve biogas production or purity, nor current density. **Given that BES perform best under high organic loading rates (OLR), the feed was supplemented with a synthetic medium, increasing the OLR from 0.30 to 0.91 g COD day⁻¹ L⁻¹.** The results are in Table 3.

Table 3. Results for each working voltage (feed = S3 + synthetic medium).

Working voltage (V)	CH ₄ rate (L/ kg SV)	CH ₄ (%)	COD removal (%)	Current density (A/m ²)	Electro-fermentation coefficient ⁽²⁾
0	0,26	62	63	-	-
0,3	0,28	67	58	0,2	0,08
0,6	0,31	68	65	0,48	0,11
0,9	0,28	66	52	0,91	0,35
1,2	0,19	64	40	1,4	1,07

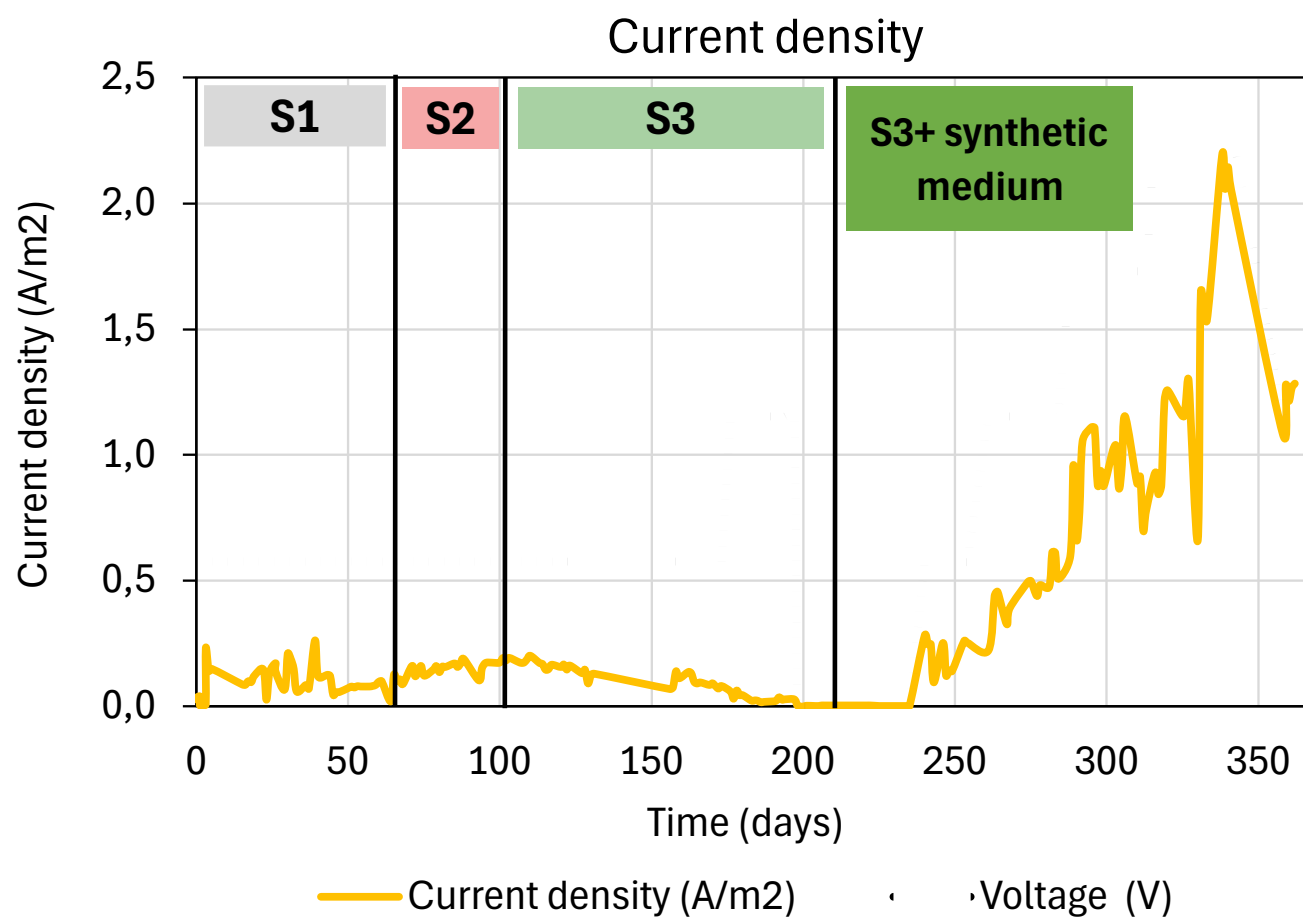


Fig 5. Current density.

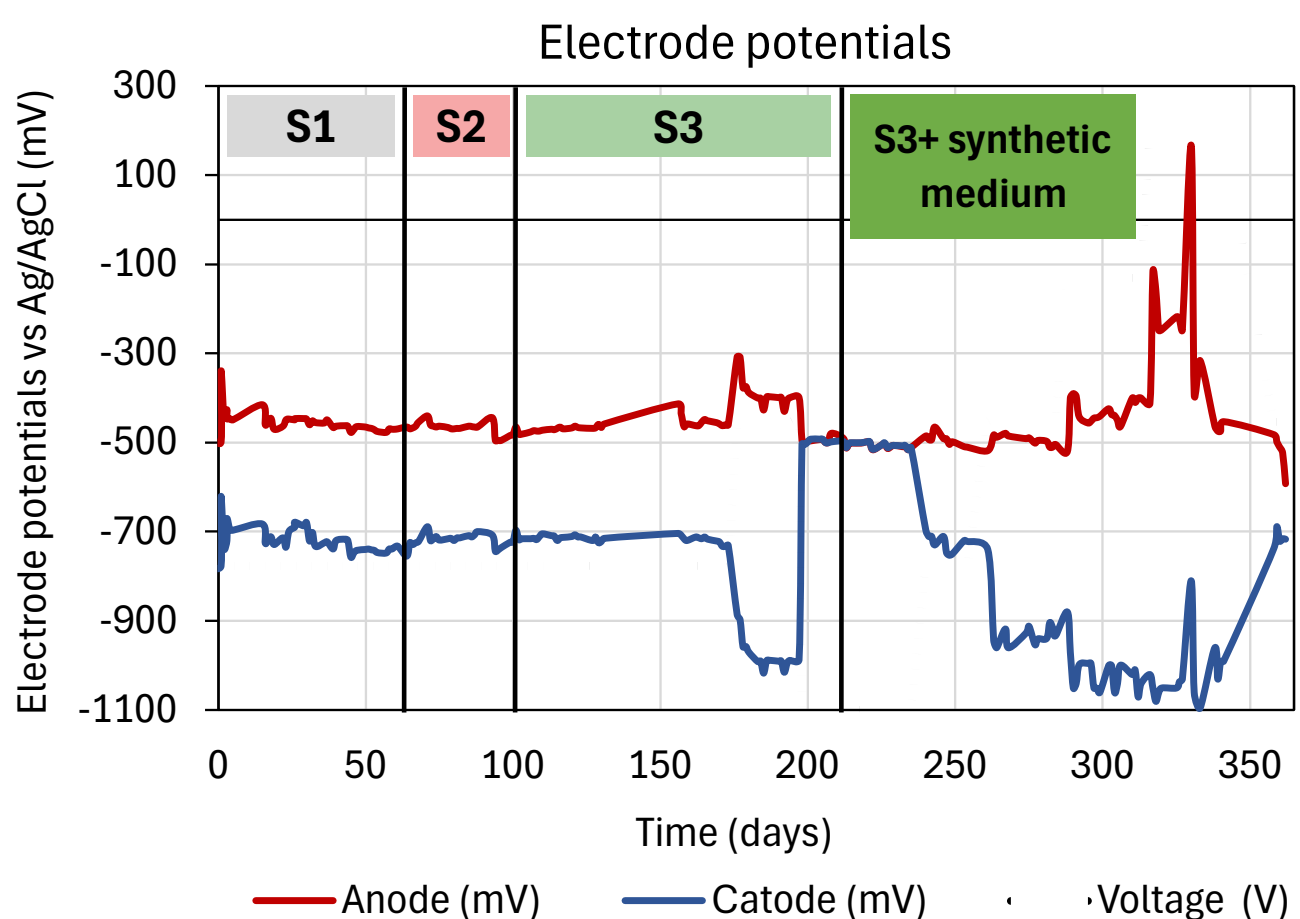


Fig 6. Electrode potentials.

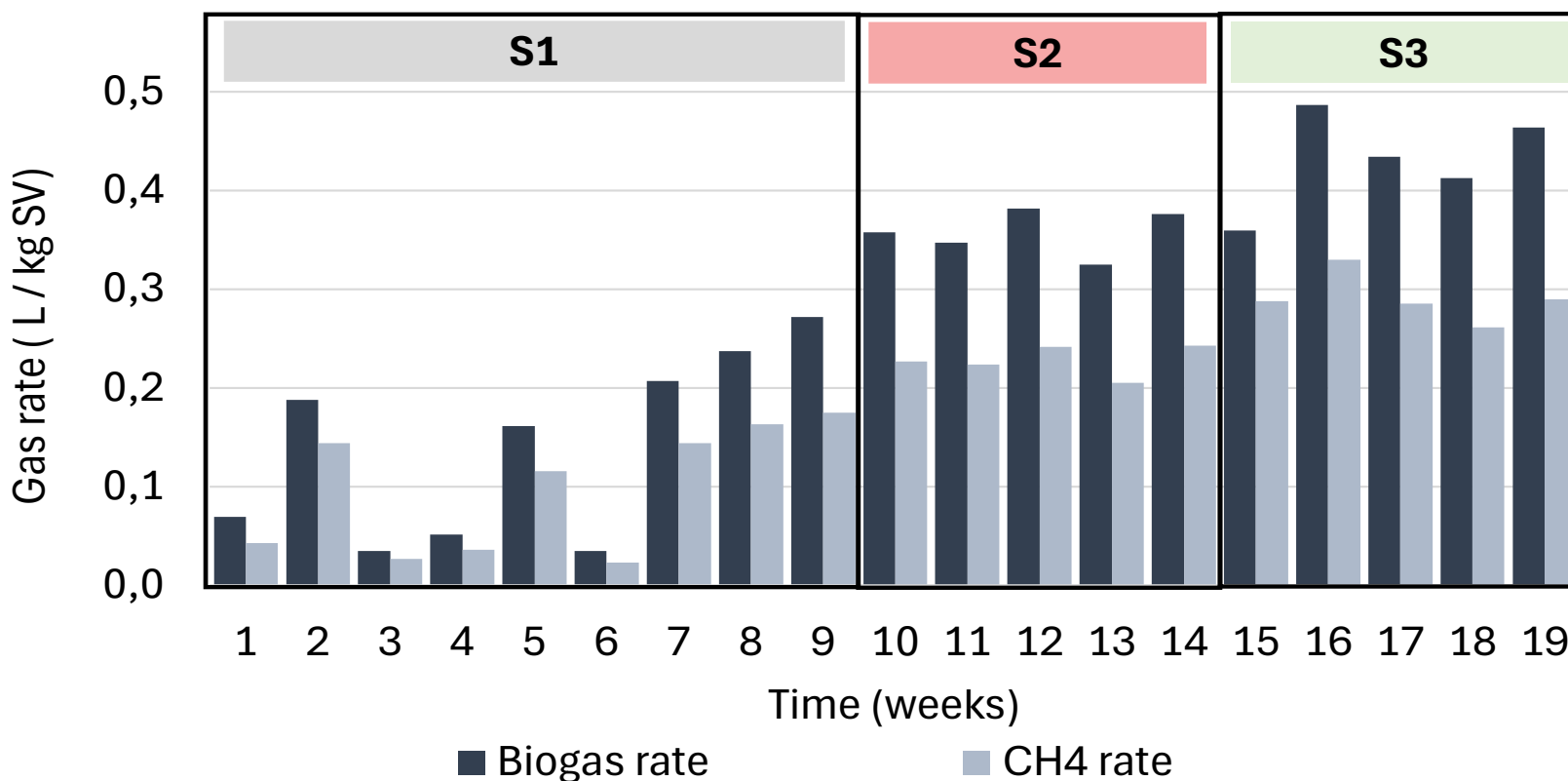


Fig 4. Biogas composition for each co-digestion phase.

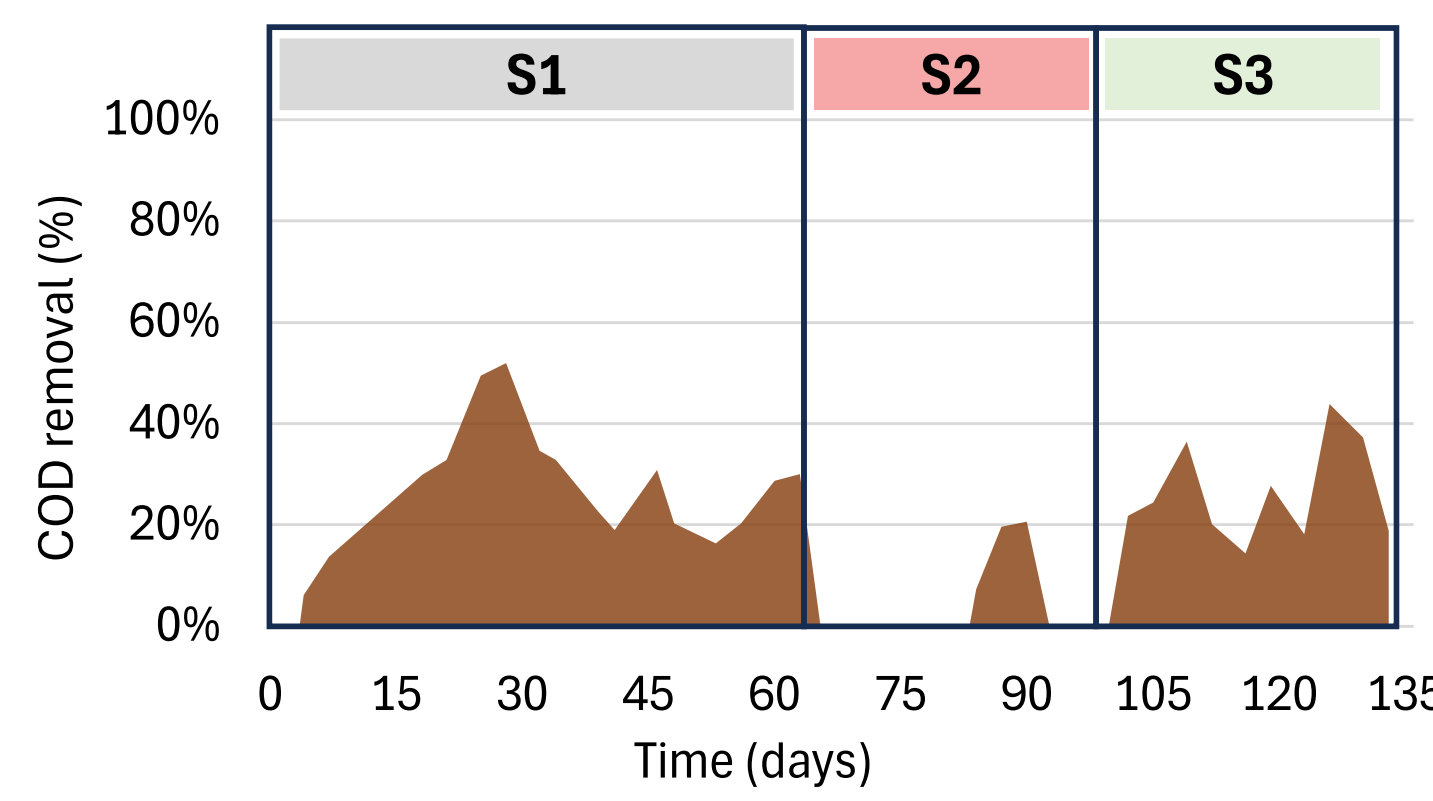


Fig 3. COD removal for each co-digestion phase.

- **Olive mill enhanced overall reactor performance and stability, making it a promising co-substrate for optimizing AD-BES reactor operation.**

At low OLR, increasing the voltage from 0.3 to 0.6 V led to a decrease in current and a rise in anode potential from -450 to -350 mV vs Ag/AgCl, indicating **substrate limitation and reduced electrochemical activity** (Fig 5 & 6). However, **under higher OLR this effect reversed**, suggesting that BES cells do influence reactor performance when sufficient substrate is available:

- Average current density and electro-fermentation coefficient rise continuously with higher voltages, but this doesn't translate into better methane production at voltages above 0.6 V.
- **Above 0.6 V (0.9–1.2 V), the anodic potential increases**, deviating from the optimal -450 mV vs Ag/AgCl.
- And **above 0.6 V** anodic potential and current density become **less stable** under these conditions.

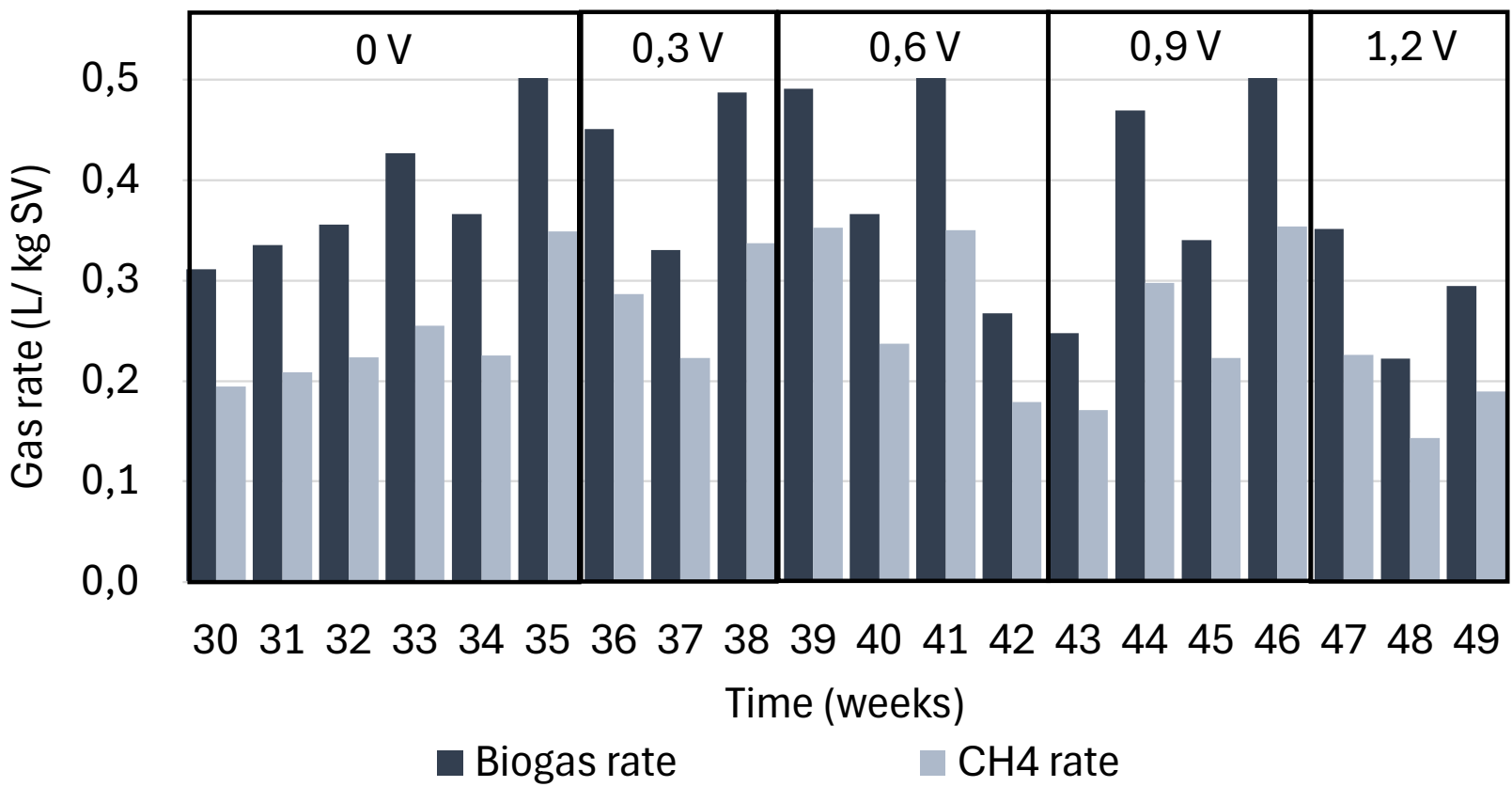


Fig 7. Biogas production in each voltage phase.

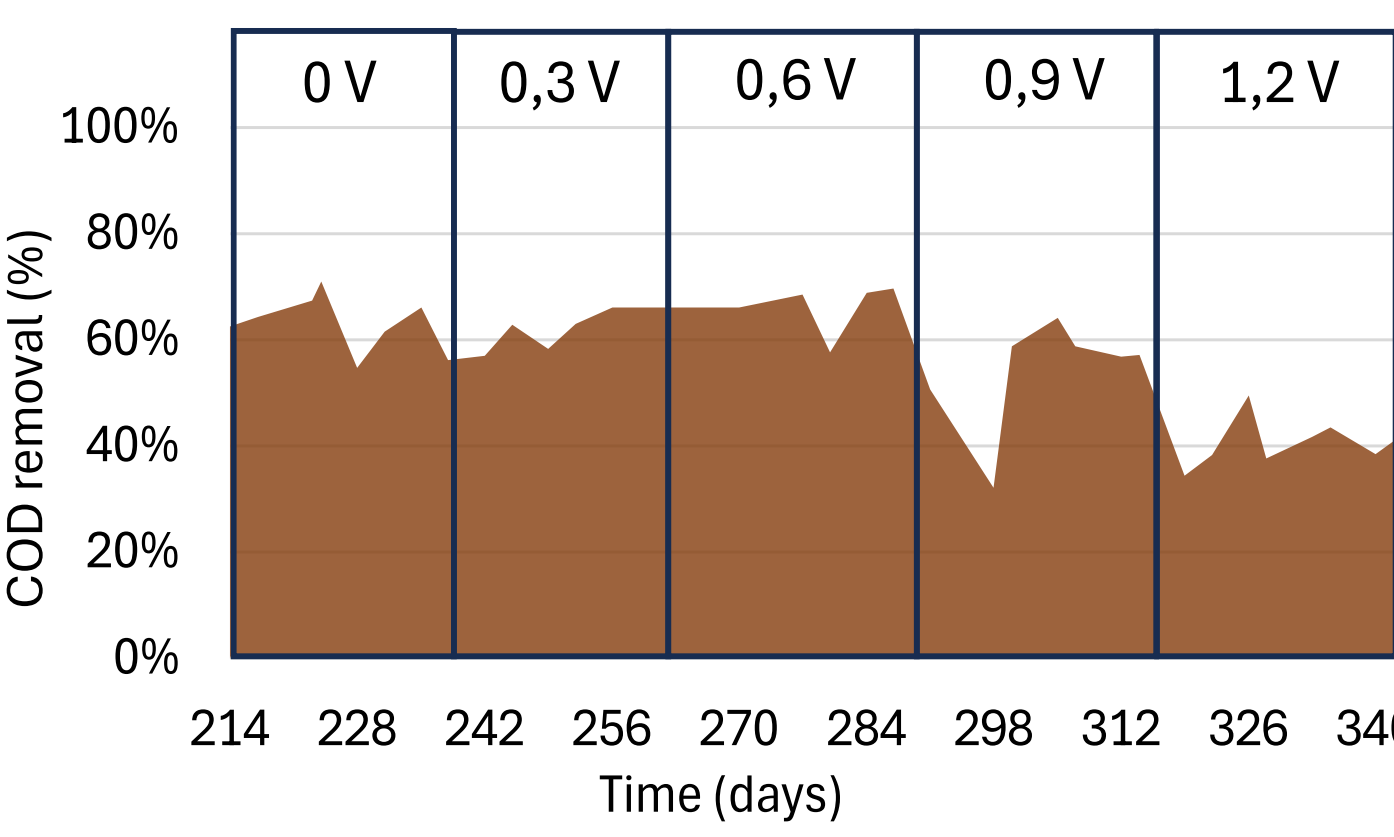


Fig 8. COD removal for each working voltage.

- **Methane production and COD removal have an optimal voltage range of 0.3–0.6 V**, beyond which benefits of electrical stimulation decrease (Fig 7 & 8).

- Higher voltages (>0.6 V) cause instability in anodic potential and current density.
- These combined effects reduce reactor efficiency, emphasizing the need to optimize voltage to maintain microbial activity and system stability.
 - ❑ Declines in performance at **voltages above 0.6 V are linked to electronic stress** damaging microbial cells and enzymes.
 - ❑ Elevated anodic potentials promote formation of **harmful reactive oxygen species**, such as hydrogen peroxide (H₂O₂), superoxide radicals (O₂⁻), and hydroxyl radicals (-OH), **affecting microbiota**.
 - ❑ Increased current densities lead to **inefficient electron transfer** and unfavorable redox shifts, impairing microbial metabolism⁽³⁾.

CONCLUSIONS

In summary, these results suggest the existence of an **optimal voltage range (approximately 0.3 to 0.6 V)** at which the AD-BES system maximizes methane production and organic matter removal, while higher voltages may compromise process efficiency. These findings are consistent with previous studies highlighting the need to balance electrical stimulation to avoid adverse effects on the anaerobic microbiota.

- **Olive mill wastewater improves** the overall performance of the **AD-BES reactor (81%)**.
- **BES technology enhances reactor stability and efficiency under high OLR.**
- Methane production and COD removal reach their maximum at voltages **between 0.3 and 0.6 V (0,31 and 58-65%)**; above this range performance decreased due to instability in anodic potential and current density.
- **High voltages cause electrochemical stress**, generation of harmful reactive species, and inefficient electron transfer, which impair microbial activity **and reduce reactor efficiency**.

REFERENCES

1. Colantoni S, Molognoni D, Sánchez-Cueto P, et al (2024) Bioelectrochemically-improved anaerobic digestion of fishery processing industrial wastewater. Journal of Water Process Engineering 65:105848. <https://doi.org/10.1016/j.jwpe.2024.105848>
2. Moscoviz R, Toledo-Alarcón J, Trably E, Bernet N (2016) Electro-Fermentation: How To Drive Fermentation Using Electrochemical Systems. Trends in Biotechnology 34:856–865. <https://doi.org/10.1016/j.tibtech.2016.04.009>
3. Cristiani L, Zeppilli M, Brutti S, et al (2024) Impact of extended starvation conditions on bioelectrocatalytic activity of a methane-producing microbial electrolysis cell. Bioresource Technology 413:131491. <https://doi.org/10.1016/j.biortech.2024.131491>

The project Waste4Soil has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No. 101112708. This output reflects only the author's view, and the European Union cannot be held responsible for any use that may be made of the information contained therein

