Pre-pilot bioelectrochemically improved anaerobic digestion of fish processing industrial wastewater

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Anaerobic digestion (AD) is a sustainable, environmentally friendly, and low-cost technology that allows the biological generation of biogas while at the same time treating organic waste. One strategy that has received attention to improve bioenergy recovery in the AD process is its integration with bioelectrochemical systems (BES) (Cerrillo *et al.*, 2018), a type of electrolytic cell where the electrodes are immersed in the anaerobic digester medium and are coated with a biological catalyst composed of microorganisms that are capable of extracellular electron transfer. In the case of the anode, electroactive bacteria oxidize small organic molecules, such as acetate, while transferring the electrons to the solid electrode. Electrons generated at the anode are transported through an external electrical circuit to the cathode, where they are utilized by hydrogenotrophic microorganisms to reduce CO_2 to methane (Villano *et al.*, 2010). The integration of a BES module in the recirculation loop of an AD reactor allows to increase the biogas production rate, process yield and resiliency of the biological process (Park *et al.*, 2020). Although BES can achieve high performances when operated in synthetic media, the situation changes when they are operated in amendment-free real wastewaters (Rosa *et al.*, 2017). In a view towards BES integration into the AD unit, is therefore necessary to investigate further its performance when operated in realistic industrial conditions which are transferable to large-scale applications. To this aim, the present study investigates for the first time, to the best of the authors knowledge, the operation of an AD-BES fed with shrimp processing industrial wastewater, a type of fishery wastewater which is considered a potential novel feedstock for biogas production (Zappi *et al*., 2019).

A 5 L anaerobic digester was connected with a side-stream stack of 5 single-chamber BES units of 0,5 L each one, utilizing carbon fiber brushes as anodes and novel 3-D printed cathodes of martensitic stainless steel 17-4PH, with a gyroid-kind internal structure. The modular BES stack was electrically connected in parallel and controlled at a cell voltage of 0.7 V, inoculated, and acclimated to the wastewater using a step-wise procedure, utilizing mixed-cultures inoculums from easily-accessible sources and a synthetic acetate medium, which was progressively switched to the real wastewater.

The AD-BES was batch-operated for more than 5 months with shrimp processing industrial wastewaters as sole carbon and energy source. Additionally, the response of the system to different perturbations was analyzed, such as operating the BES modules in open circuit voltage (OCV), shutting down the complete system and progressively disconnecting the BES modules from the main AD reactor. At the end of the experimentation, a microbiological analysis was carried out on the anodes, cathodes, BES electrolytes and AD digestate to identify microbial differences and similarities between the electrodes and the liquid phases. From the overall results shown in Figure 1, we can observe an efficient process for shrimp wastewater treatment with a high efficiency in chemical oxygen demand (COD) removal (85-93%, corresponding to a COD of the discharged effluent around 1 g/L) and biogas production (ranging from 300 to 900 L_{CH4} $m^{-3}_{reactor}$ day^{-1}, corresponding to a methane yield in the range of 400-600 L_{CH4} kg^{-1}_{COD}), both achieved at low hydraulic retention times (9-12 days). The OCV test had no significant effects on biogas and methane yield and COD removal, which remained near 85%. The shut-down test determined a residual biogas production capacity of the plant of 700 L_{biogas} $m^{-3}_{reactor}$ with a complete stop of biogas production achieved in 90 hours.

The progressive disconnection of BES modules from the AD did not influence the biogas production nor its composition (methane content) within individual batch cycles. However, it was possible to observe an increase in volatile fatty acids (VFA) levels and a decrease of both electrical current and COD removal efficiency after disconnecting the 3rd module. Reconnecting all the BES modules determined a decrease of VFA and an increase of COD removal. These results could indicate that the beneficial effect of the BES on the AD should be investigated more on the process control, resilience to perturbation and/or operation at high OLR rather than on the improvement of the biogas production rate and composition.

Analysis of the microbial community by 16S rRNA metabarcoding revealed the predominance in all reactor parts of genera *Proteinclasticum*, *Pseudomonas*, *Gallicola* and *Christensenellaceae_R-7 group*, which are well-known fermenting bacteria that promote hydrolysis and VFA production (Fang *et al*,. 2022). Moreover, the community composition in the electrodes differed compared to the liquid phase, increasing the abundance of the genera *Thermovirga*, *Romboustia*, *Clostridium sensu stricto* that may contribute to electron flow in syntrophic methanogenesis, by working as Syntrophic Acetate Oxidizers (SAO) (Charalambous *et al*. 2020).

A 1 m^3 pilot-plant of AD-BES, based on the pre-pilot laboratory configuration, will be started-up in an industrial demo-site producing fishmeal and fish oil (Prima Protein, Eigerøy, Norway) during the beginning of 2024. The start-up and operation of the pilot plant will bring novel information on the feasibility of AD-BES application in real industrial processes and on the optimal process parameters for such a system.

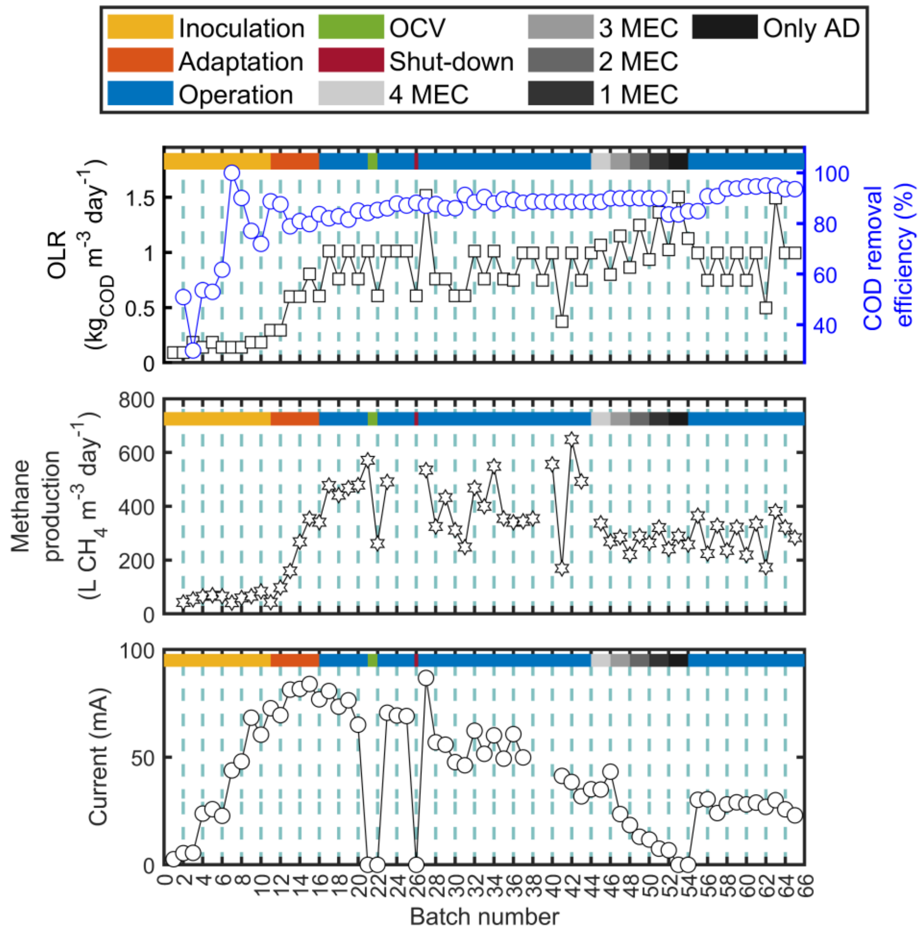

Figure 1. From up to down: trend of the organic loading rate (OLR) during the experimentation, trend of the methane production rate during the experimentation and average electrical current (sum of the currents of all the MEC modules operating) for each batch. The line connecting the points serves only to guide the eyes. The colored line on top of each graph visually indicates each experimental phase.

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