Journal Pre-proofs

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PII: S1385-8947(22)05307-4
DOI: https://doi.org/10.1016/j.cej.2022.139828
Reference: CEJ 139828

To appear in: *Chemical Engineering Journal*

Received Date: 2 August 2022
Revised Date: 23 September 2022
Accepted Date: 12 October 2022

Please cite this article as: C. Manchon, F. Muniesa-Merino, D. Serna, Y. Asensio, C. Wardman, A. Esteve-Nuñez, Fluid-like electrodes and Purple Phototrophic Bacteria: bridging the gap in wastewater biorefineries, *Chemical Engineering Journal* (2022), doi: https://doi.org/10.1016/j.cej.2022.139828

Fluid-like electrodes and Purple Phototrophic Bacteria: bridging the gap in wastewater biorefineries

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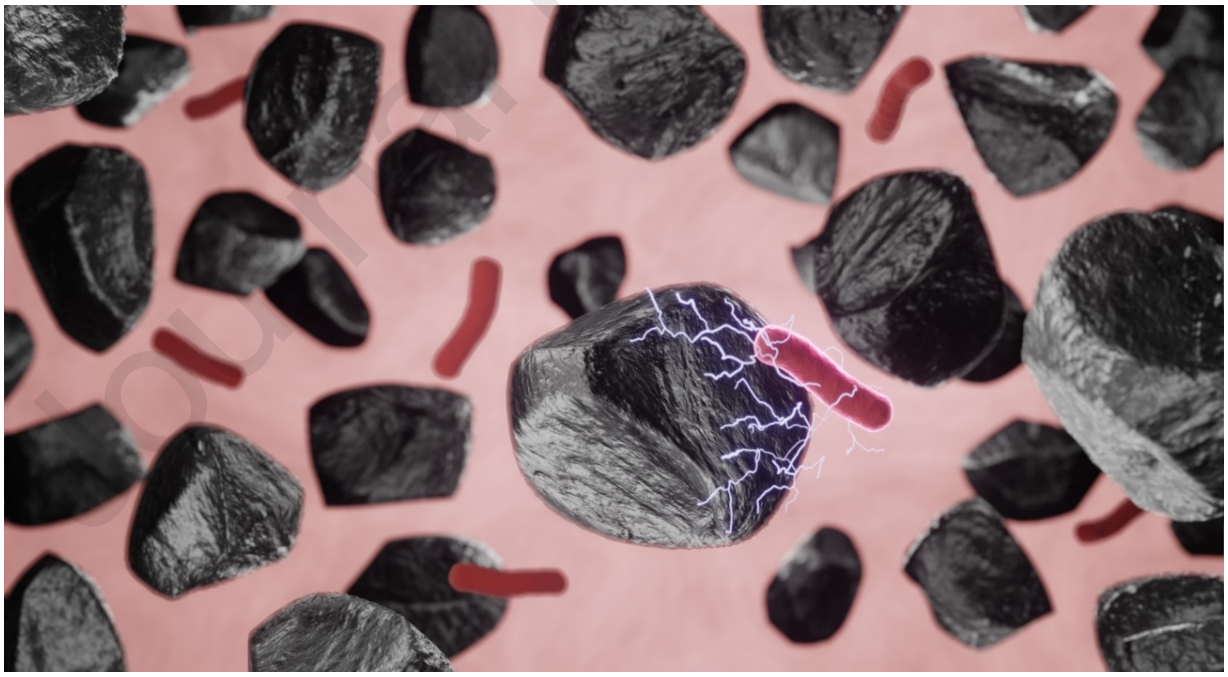
Abstract

Wastewater biorefineries aim to generate value-added products in an economically viable process while removing pollutants. In this scenario, Purple phototrophic bacteria (PPB), the most versatile microorganisms on earth, are highly effective for sustainable wastewater treatment and nutrient recovery as cell protein. One of the most innovative approaches for applying PPB in the wastewater sector is their capacity for interchanging electrons with electroconductive materials. In contrast with classical biofilm-based techniques, we have demonstrated that a fluid-like electrode can accept electrons from planktonically grown PPB. We anticipate that such findings will impact in wastewater electrobioremediating capacity of PPB. Moreover, controlling the electrochemical nature of the extracellular electron acceptor (fluid-like electrode) allows for fine-tuning the metabolism of a planktonic PPB-dominated community to enhance their biodegradation rate (2-fold) while growing on brewery wastewater. For this purpose, a twin set of microbial electrochemical fluidized bed reactors (ME-FBR) were operated in identical conditions, except for illumination conditions (dark vs. infrared), to promote the development of PPB. Illumina sequencing revealed that both infrared radiation and polarization led to changes in the microbial population while producing an electrical current of 7 $A \cdot m^{-3}$. Indeed, the *Geobacter* genus was the electroactive bacteria outcompeting under dark conditions. In contrast, electroactive PPB genera like the *Rhodopseudomonas* and *Rhodobacter* outcompeted others under infrared illumination and electrostimulation. In this work, we have demonstrated how microbial selection can contribute to the sustainability of an electrobioremediation wastewater treatment by avoiding emissions of greenhouse gases such as methane. In addition, fluid-like bed bioreactors have shown their usefulness in recovering nutrients as PPB biomass, favoring planktonic growth and thus facilitating the recovery of a valuable product: the biomass of PPB.

Graphical abstract



Introduction

For more than a century, activated sludge has treated wastewater. This process has improved worldwide quality of life and health while reducing the environmental impact of eutrophication [1]. The increasing demands of the climate crisis require rethinking wastewater treatment. It is no longer enough only to remove pollutants; to address the coming crisis, places like wastewater treatment plants (WWTPs) also need to recover nutrients and energy into

viable products or resources for reuse [1]. To achieve this goal, the underlying concepts of the WWTP need to be reimagined into a wastewater biorefinery concept

Purple Phototrophic Bacteria (PPB) are considered one of the most metabolically versatile microorganisms on earth[2]. These microorganisms have been found in very different ecosystems, including those with extreme pH and temperature, or environments polluted by recalcitrant compounds. Its ubiquity is due to its metabolic versatility, using infrared light (IR) and a wide variety of organic compounds as its main energy and carbon source[2]. These versatile characteristics allow them to grow photoheterotrophically under anaerobic conditions while using light as their energy source. Thus, PPB can simultaneously assimilate carbon and nutrients at high efficiency [3], facilitating the maximum recovery of these resources as bioplastics[4] , biohydrogen[5] and cellular biomass. The high protein content of PPB biomass makes it a promising product as single cell protein, which has been tested as a feed additive and bulk ingredient in aquaculture[6,7]. Despite its promising potential in recovering nutrients from wastewater, mixed culture products of PPB are not yet commercialized [8].

PPB are capable of exchanging electrons with extracellular electron donors and acceptors, such as iron and manganese oxides[9]. This capacity allows PPB to have a redox interaction with electroconductive materials, like electrodes[5,10–12], making them suitable for performing microbial electrochemistry. In processes such as wastewater treatment, where diverse microbial populations meet complex mixtures of organic compounds, electrodes become a control and stabilization element. They can help to overcome metabolic limitations or metabolic imbalances through a process called Electro-Fermentation (EF)[13].

In conventional microbial electrochemistry, solid electrodes (eg. rods, plates, and felts) are typically used as electroconductive materials[14] to support biofilm growth. Under such conditions diffusion and migration processes become a limiting factor for achieving optimal biodegradation rates. In contrast with such static electrodes, an innovative electrochemical configuration called the microbial electrochemical fluidized bed reactor (ME-FBR) uses a fluid-like electrode to minimize mass transfer and energy ensuring proper mixing inside the reactor. Furthermore, the fluid nature of the electrode allows, in addition to the biofilm-based interaction of static electrodes, interaction through contact with planktonic cells. [15]. Indeed, a fluid-like anode has been shown to be efficient for removing organic pollutants and nitrogen from industrial brewery wastewater[15,16] Furthermore, a fluid-like electrode serves as the sole electron donor for promoting microbial denitrification in an organic carbon-depleted medium[17]. Additional studies using alternative mobile electroconductive beds have confirmed how bacteria can charge such material with electrons from their metabolism[18–20].

However, the biotechnological potential of PPB in wastewater treatment and nutrient recovery is limited using current electrochemical tools constrained by the requirement of biofilm growth. Thus, in this work, we have overcome such a bottleneck by demonstrating how a fluid-like electrode stimulates PPB growth under planktonic conditions while enhancing assimilative metabolism and cell yield to electrobioremediate brewery wastewater to generate a valuable product: nutrient-rich PPB biomass. Therefore, our approach combines an efficient treatment of brewery wastewater with the generation of a value-added product, thus bridging the gap in the transition from classical wastewater treatment model to a sustainable biorefinery model.

Material and methods

Experimental Set-up and operating conditions

Two ME-FBR units were built following the design described in Tejedor-Sanz et al, (2018)[15]. The reactors were made of borosilicate with a total volume of 0.1 L. Using a three-electrode cell system, the reactors featured a fluidized working electrode (WE), a reference electrode located near the working, and a counter electrode (CE) located at the top of the reactor. Vitreous carbon (20 mL, 0.6 – 1 mm diameter) (Sigradur G, HTW, Germany) was used as fluidized anode (WE) and a graphite rod (Mersen, Spain) was immersed in the bed as a current collector. Platinized titanium mesh (Inagasa, Spain) was used as a cathode (Counter electrode). A 3 M KCl Ag/AgCl reference electrode (Hanna) was used as a reference electrode.

The fluidization of the bed was achieved through the recirculation of liquid by means of a peristaltic pump (Heidolph 5006, Germany) at a flow rate of 200 $mL \cdot min^{-1}$ (0.11 $cm \cdot s^{-1}$ linear velocity). A flow distributor was in the lower section of the reactor to ensure optimal flow sharing and fluidization.

One of the reactors was operated under infrared illumination (IR) conditions and the other one was operated under dark conditions as a control (Fig 1). We used the same operating conditions for both reactors (influent, inoculum, and polarization potential). The reactors were inoculated (1:10 (v/v)) using sludge from anaerobic digester IWWTP. The reactors were operated with an open circuit potential as the acclimatization stage until steady-state conditions (in terms of chemical oxygen demand (COD) removal and optical density (OD)) was reached. The reactors were then operated under two polarization values until current densities were stable and steady state conditions were achieved. The bed was polarized first at 0.2 V, then at 0.4 V and finally it was operated under open circuit polarization (OCP). The OCP results shown in this work correspond to the experimental period after polarization.

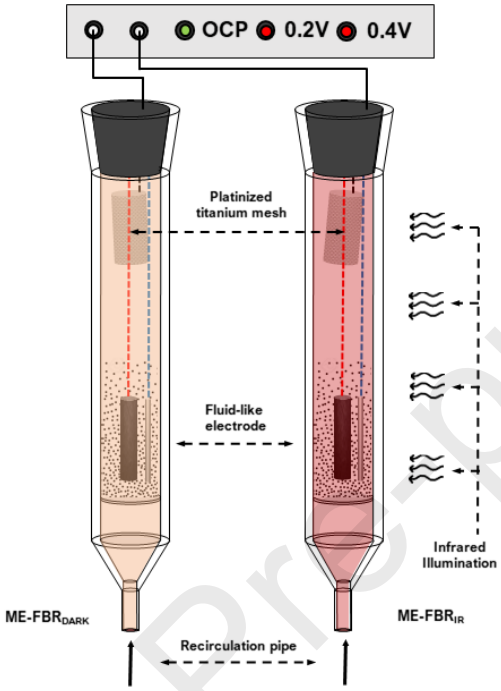


Figure 1. ME-FBR scheme. Left: ME-FBR$_{DARK}$. Right: ME-FBR$_{IR}$. Wavy arrows indicate infrared radiation. The medium was recirculated from the upper part of the reactors to the lower part to fluidize the bed.

We also evaluated the effect of only the electroconductive material on the performance of the ME-FBR$_{IR}$ reactor and biocompatibility regarding PPB growth yield. Thus, two reactors were operated under infrared lighting conditions with synthetic wastewater with acetate (40 mM) as sole electron donor and carbon source[21]. The second control reactor was used for growing PPB consortium in the absence of any fluidized material (Fig 1 SI).

Chemical analyses

Organic contaminants were analyzed using Total Organic Carbon (TOC) and Chemical Oxygen Demand (COD) after filtering (0.22 micrometer). TOC was measured by a TOC-VCSH Shimadzu analyzer. COD was quantified using a commercial kit (Merck Millipore, Germany) as previously described[16,22]. Acetate was analyzed by HPLC (HP series 1100, UV detector 210nm and Supelco C-610H column). The theoretical COD for synthetic wastewater was calculated from acetate HPLC determination as previously described[23]. Ammonium, nitrate, and nitrite were analyzed by ionic chromatography (Metrohm 930 Compact Ion Chromatograph Flex), for which they were filtered at 0.45 µm and later at 0.22 µm with a tangential filter. Methane headspace concentration was measured by gas chromatography (Varian 3350 chromatograph with a packed column, Porapack N 80/100) with nitrogen as the carrier gas (20mL·min^{-1}) and a thermal conductivity detector.

Microbiological analyses

PPB growth was analyzed by measuring the absorption peaks of bacteriochlorophylls and carotenoids[5] in the wavelength range of 1100 to 400 nm (Shimadzu UV-1800 Spectrophotometer). The growth curve data corresponds to the absorbance at 590 nm to minimize measurement error[24].

16S Microbial community analysis was performed following the Illumina protocols and Miseq equipment. Illumina Basespace (16S) software was used for data analysis (Autonomous University of Barcelona, Spain). The v3 and

v4 regions of the 16S rRNA gene were amplified using the primers: 16S Amplicon PCR Forward Primer = 5' (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG) and 16S Amplicon PCR Reverse Primer = 5' (GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC).

Electrochemical measurements and characterization

The fluidized reactors were polarized using a potentiostat (NEV-4 v.2, Nanoelectra S.L., Madrid, Spain). Electric current and electric potential were measured every second. Current density values were given per net volume of reactor (NRV) (**Eq. 1 SI**). Cyclic voltammetry (CV) was carried out in fluidized bed reactors at the end of the experiment with a scan rate of 10 mV · s^{-1} between 0.8 V and -0.8 V (*vs*. Ag/AgCl). Abiotic CV was performed with filtered (0.22µm) brewery wastewater to generate cell-free solution.

Moreover, to study the electrochemical response of our microbial consortium, we grew a PPB biofilm on a carbon rod using a conventional electrochemical cell in a three-electrode configuration. The electrochemical system was completed by a platinized titanium as a counter electrode, and a leakless reference electrode. The system was polarized at 0.2 V (vs Ag/AgCl) and inoculated with ME-FBR_{IR} planktonic bacteria to promote biofilm formation (3 weeks). The microbial electrochemical cell was fed daily with fresh culture medium with acetate as a carbon source.

Results

Inspired by the potential of purple phototrophic bacteria (PPB) in key environmental biotechnologies, we have explored their capacity for interchanging electrons with electroconductive materials. For the first time, we have combined infrared radiation with the operation of microbial electrochemical fluidized bed reactor (ME-FBR_{IR}) for growing electroactive planktonic PPB in real brewery wastewater (**Table 1SI**).

Fluid-like electrode and IR illumination drives changes in microbial population

Environmental conditions determine the structure and activity of a microbial community and the presence of a polarized electrode acting as electron acceptor is no exception. Thus, electrode polarization typically triggers two knock-on effects in microbial processes. First, electrodes select for specific microbial phenotypes leading to a shift in the microbial population composition. Second, polarized electrodes allow for unique metabolic activities, leading to changes in gene expression from individual community members[25,26]. This microbial population shift takes place in microbial electrochemical systems[27,28] because microorganisms capable of taking advantage of the extra electrons donors or acceptors will eventually outcompete the others. Therefore, biodiversity analysis using Illumina 16S sequencing was the first stage to explore the structure of our microbial community.

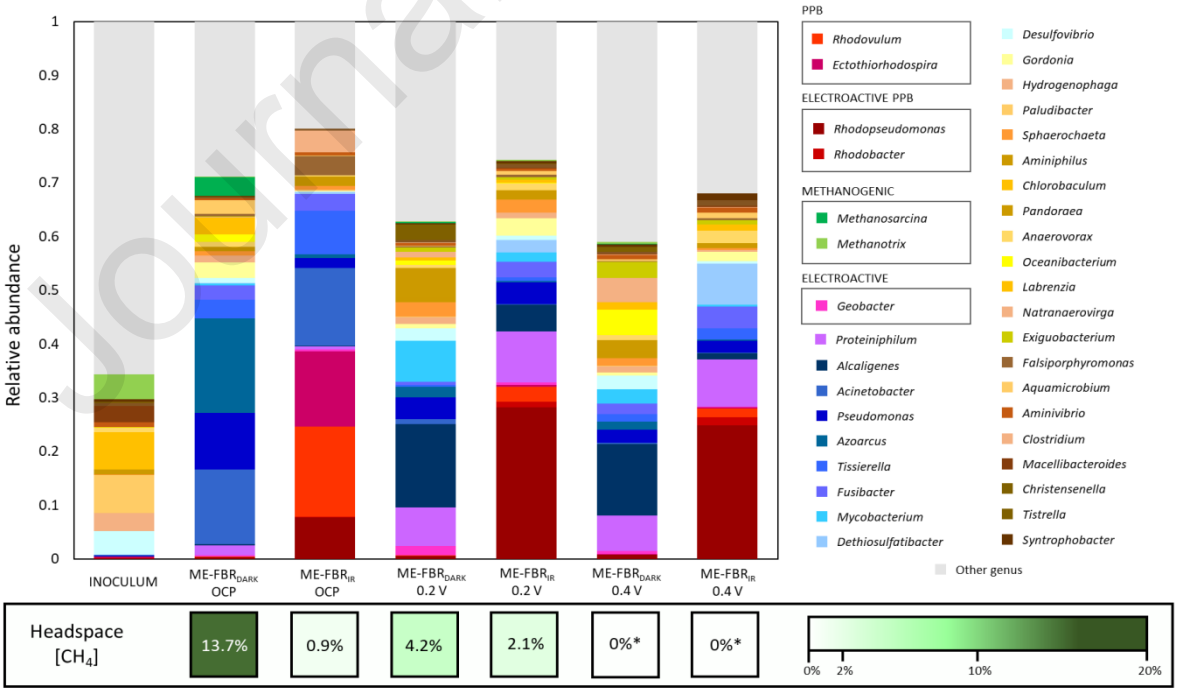


Figure 2. Relative abundance at the genus level. The legend boxes highlight the key genera that play a role in ME-FBRs. The heatmap shows the methane concentration in the reactor headspace for each of the conditions. Genera framed in methanogenic belong to the archaea domain.

We analysed the microbial community from brewery wastewater sludge used as the inoculum for our reactors. This sludge was characterized by a high content of organic compounds (mainly volatile fatty acids (VFAs)) and nitrogenous compounds but was limited in electron acceptors such as nitrate or sulphate. The analysis showed genera mostly of anaerobic and aerobic fermenters (**Fig. 2**). Moreover, some methanogenic activity was expected due to the presence of *Methanotrix*, a genus of methanogenic archaea[29].

The evolution of this inoculation community was explored while biodegrading real brewery wastewater using twin ME-FBR operated under either dark or IR illumination in the presence of fluid-like polarized anodes (at 0.2V and 0.4V vs Ag/AgCl) and non-polarized as an open circuit (OCP). Such six independent operating conditions were grouped into five clusters of variance based on Principal Coordinates Analysis (PCoA) (**Fig. 3 SI**): 1) wastewater, 2) OCP_{DARK}, 3) OCP_{IR}, 4) electrode polarization under dark conditions and 5) electrode polarization under IR conditions. These results revealed that both polarization and infrared radiation affected the population structure. In addition, the clear separation of the polarized samples (clusters 4 and 5) indicated that, under illumination conditions, the polarization exerted different selective pressures.

We observed several differences at all taxonomic levels, but specially at the genus level. We focused on those key genera previously described by other authors regarding their role: classical genera described as electroactive bacteria[30], PPB [2] and methanogenic bacteria[2,31].

The mere presence of a fluid-like electrode allowed electroactive bacteria (mainly from the *Geobacter* genus) to outcompete other species, even under open circuit conditions where electrical current cannot flow (**Fig. 2**). This process of Conductive-mediated Interspecies Electron Transfer has been recently named as CIET [32]. It is the first time this phenomenon has been described using a fluid-like electrode, although it was previously observed in a fixed electroconductive beds[33]. Furthermore, in the OCP_{DARK} reactor we also observed the prominent presence of methanogenic bacteria from the *Methanosarcina* genus. The direct extracellular electron transfer between species of the *Methanosarcina* and *Geobacter* genus has been widely demonstrated[34,35]. We hypothesize that fluid-like electrode can mediated such extracellular electron transfer, favouring the syntrophic relationship, and therefore promoting the presence of both genera. This was consistent with the high methane concentration detected in the headspace (13.7%), the highest level of all the conditions tested in our study (**Fig. 2**). In contrast, the non-polarized illuminated reactor (OCP_{IR}), had more PPB, specifically from *Rhodopseudomonas*, *Rhodovulum,* and *Ectothiorhodospira* genera. Interestingly, we found a significant lower abundance of those classical electroactive bacteria previously observed in OCP_{DARK}. This result suggested that infrared radiation, as an extra source of energy, promoted the presence of PPB over classical electroactive and methanogenic bacteria.

To polarize or not to polarize the fluid-like electrode

Next, the $ME\text{-}FBR_{DARK}$ was operated under polarized conditions (0.2 V and 0.4 V), revealing a higher abundance of bacteria from *Geobacter* genus compared to OCP_{DARK} (**Fig. 2**). These results confirm previous studies where a fluid-like electrode, acting as a terminal electron acceptor (TEA), promotes the development of electroactive bacteria. This was shown using FISH techniques by the presence of electroactive bacteria at the inner layers of the biofilm, probably acting as a connector between other bacteria genera and the electrode[15]. In addition, we observed a significantly lower abundance of methanogenic species when the bed was polarized in comparison to OCP_{DARK}. These results and the low methane concentrations detected in the headspace (**Fig. 2**) suggested that the electrode is a true TEA competing for electrons and minimizing or fully avoiding methanogenesis. We hypothesize that, under dark conditions, *Geobacter* acts as a connector in the electrically conductive fluidized bed, transferring electrons either to i) the electrode when the fluid-like anode is polarized and ii) to the methanogenic partners when the fluid-like anode is non-polarized (OCP).

In the case of the polarized and illuminated bioreactor $ME\text{-}FBR_{IR}$, we also observed a high relative abundance of PPB (**Fig. 2**). In contrast to the OCP_{IR}, the majority genera present in the polarized $ME\text{-}FBR_{IR}$ were *Rhodopseudomonas* and *Rhodobacter*. These two types of PPB have been reported to be electroactive[10,36,37], so their ability to interact with the electrode could justify why polarization selects for them.

We must emphasize the importance of the genus *Proteiniphilum* in polarized reactors, under both dark and light conditions. This genus has been actively selected for in other bioanodes systems fed with complex organic compounds, such as wine industry wastewater[38,39]. It is considered a partner of electroactive bacteria, metabolizing complex molecules to oxidizable metabolites for them[40]. Our results suggest that in polarized $ME\text{-}FBR_{DARK}$, *Proteiniphilum* species could establish symbiosis with *Geobacter*, while in $ME\text{-}FBR_{IR}$, it would act as a partner of electroactive PPB such as *Rhodopseudomonas* and *Rhodobacter*.

Fluid-like electrode enables photo-electro-fermentation

The interaction between the microorganisms and the fluid-like electrode can be monitored through current density, so the actual role of the electrode in the microbial process can be electrochemically explored. Regardless the absence or presence of illumination, electric current production was always detected during the operation of both reactors. The volumetric current density of ME-FBR$_{DARK}$ (max value of 40 A·m^{-3}) was higher than the one measured in the ME-FBR$_{IR}$ (max value of 7 A·m^{-3}) (**Fig.3**).

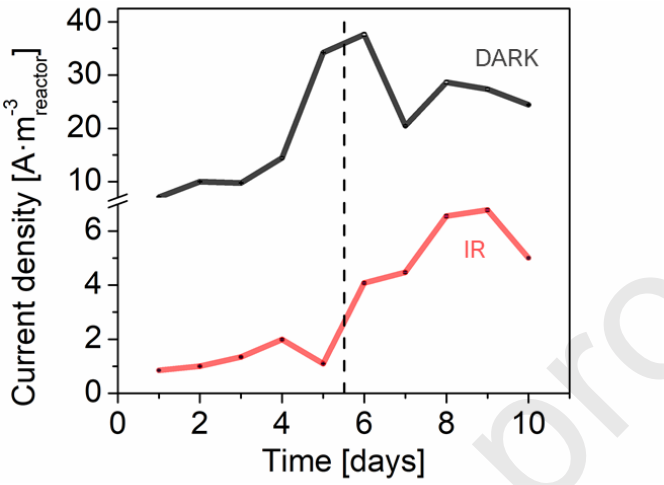


Figure 3. Current density in the fluidized anode polarized to 0.2V (0-5 days) and 0.4V (6 to 10 days).

As typically occurs in Microbial electrochemical systems just a percentage of electrons from microbial oxidation of organic pollutants were harvested as electrical current. We observed coulombic efficiencies (CE) of about 22% at 0.2 V and 41.0% at 0.4 V for the ME-FBR$_{DARK}$, confirming that fluid-like anode acts as terminal electron acceptor for bioremediating pollutants. These coulombic efficiency values suggested that the electrode was not acting as the sole electron acceptor in the oxidation processes. The detection of methane confirms the role of alternative electron sinks (**Fig. 2**). These CE values were similar or even slightly higher than those previously reported using ME-FBR$_{dark}$ [15].

The illuminated reactor (ME-FBR$_{IR}$) had considerably lower CE values (1.1% at 0.2 V and 4.1% at 0.4 V) in comparison with the ME-FBR$_{DARK}$. The metabolic versatility of PPB, predominant in illuminated reactor, allows them to use alternative metabolic pathways such as nitrogen and carbon fixation or hydrogen production as electron sink[41]. Such electron-sink pathways make PPB less dependent on an extracellular electron acceptor like an electrode, which is consistent with the low CE values.

Two-way extracellular electron transfer in PPB consortium

To understand the role of the electrode on PPB metabolism and how it modulates the activity of such microorganisms, we explored bacteria-electrode interaction by cyclic voltammetry analysis. We carried out the

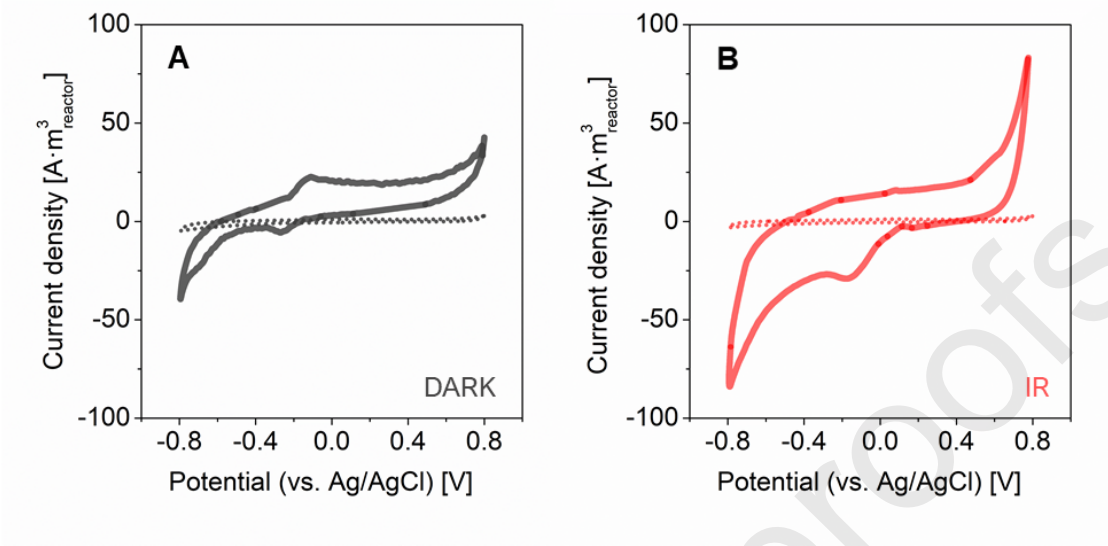


Figure 4. Steady-state voltammograms of ME-FBR at the end of the operation using brewery wastewater: ME-FBR$_{DARK}$ (A) and ME-FBR$_{IR}$ (B). The discontinuous voltammogram corresponds to the abiotic conditions. Scan rate: 10 mV/s. The figure shows the third cycle after steady state was reached

electrochemical analysis in two different situations: i) electron transfer from planktonic PPB to a fluid-like electrode and ii) electron transfer from a PPB-based biofilm to a rod-like electrode. Performing CV directly on the ME-FBR allows for the *in situ* study of the planktonic bacteria-electrode interaction. On the other hand, a biofilm-based system is a more stable scenario for accurately investigating the electrode's role in the regulation of cellular metabolism and for establishing a comparison with previous electrochemical studies using PPB[42].

The results obtained in ME-FBR showed a different light-dependent electrochemical behaviour (**Fig.4**). In the ME-FBR$_{DARK}$, the appearance of a redox couple was observed in comparison with the abiotic voltammogram. These redox peaks revealed an electrochemical interaction between the microbial consortium and the fluid electrode. The midpoint potential of the redox pair (-0.19 V *vs.* Ag/AgCl) was similar to those obtained with pure cultures of species of the genus *Geobacter*[43]. Furthermore, we observed a sigmoidal shape that suggests catalytic biological activity. The voltammograms corresponding to ME-FBR$_{IR}$ showed a non-reversible reduction peak at -0.16 V (*vs.* Ag/AgCl) and, in contrast to the ME-FBR$_{DARK}$ results, we did not observe any oxidation activity. These results were consistent with the low coulombic efficiencies observed and could indicate that in the illuminated reactor, the fluid-like electrode is not acting as a sole electron acceptor. It is worth highlighting that the reduction activity observed in the voltammogram could be related to reduction processes catalysed by PPB reported elsewhere[10,44].

The fluidized bed allows the electrode polarization to be more homogeneous in comparison to fixed bed systems[15,45]. However, the internal resistance of the bed is still too high to precisely control the potential for performing accurate CV[46]. Therefore, using PPB community grown in the ME-FBR$_{IR}$ as inoculum, we grew a PPB biofilm on a standard rod graphite electrode to give insights into the possible Extracellular Electron Transfer (EET) mechanism, and on metabolic processes.

Three redox pairs (RP1, RP2 and RP3) were clearly identified from cyclic voltammetry in the absence of acetate, under nonturnover (NTO) conditions. These redox-active compounds are possible EET sites, which could connect the electrode with bacterial metabolism to control different bioelectrochemical processes (**Fig. 5**).
Classically, in microbial electrochemistry, the oxidation of organic compounds has been considered as the main anodic process[47]. However, other anodic processes like electrode-dependent anaerobic ammonium oxidation[48] has been recently described. To identify those real EET sites capable of regulating bioelectrochemical anodic processes we proceeded to investigate the metabolism of different substrates: acetate and ammonium. The CVs under turnover (TO) and nonturnover conditions revealed that the Ef1 was the EET site related to the oxidation of acetate (**Fig. 5B**). The electroactivity of the PPB-based biofilm was confirmed by the oxidative catalysis observed in the TO voltammogram, together with an increase in electrical current when medium was spiked with acetate (**Fig. 4 SI**).

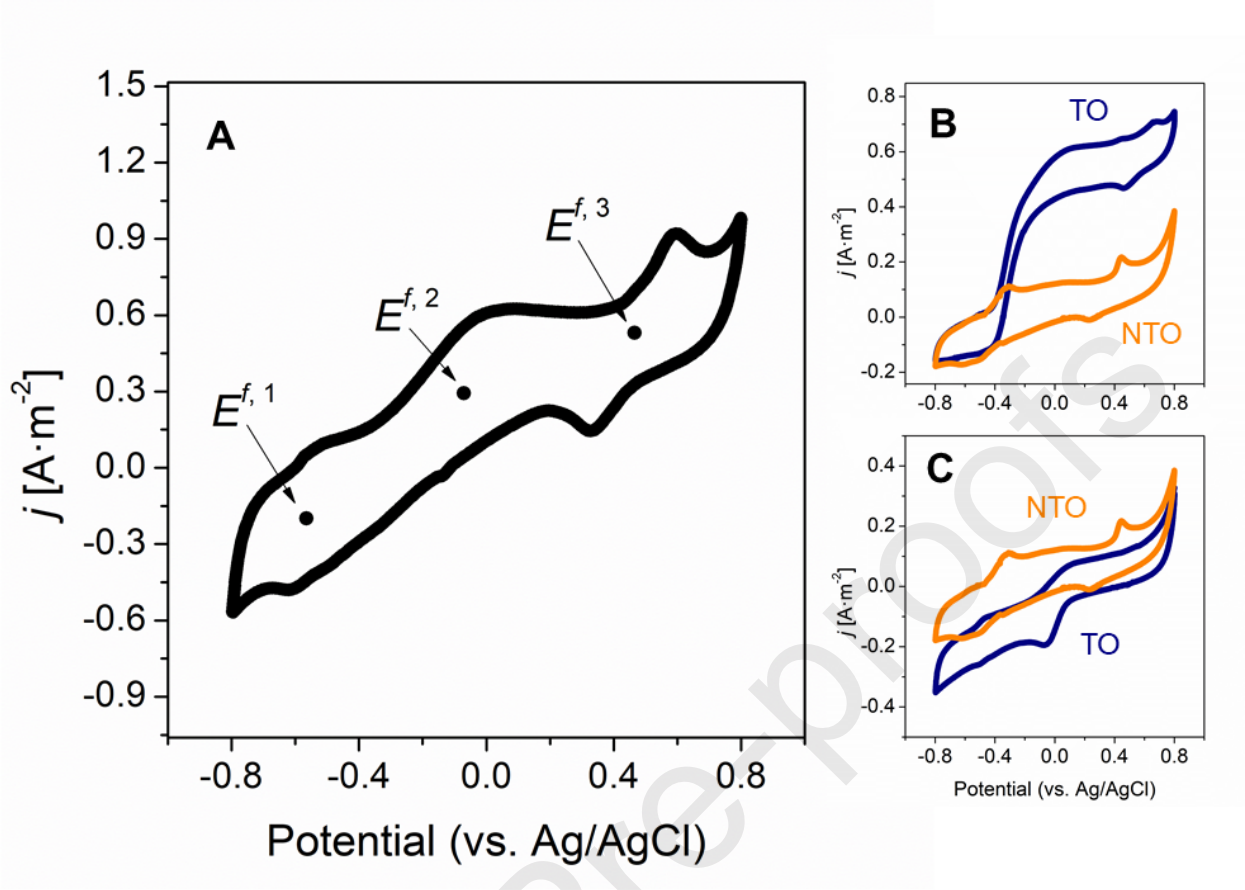


Figure 5. Steady-state voltammograms of the PPB biofilm-based system using mineral medium. **A**: Voltammogram under non turnover conditions at a scan rate of 10 mV · s^{-1}. Ef1, Ef2 and Ef3 indicate the formal potentials of the redox peaks found. **B**; Voltammogram under non turnover (orange line) and turnover (blue line) with acetate as substrate (Scan rate: 1 mV · s^{-1}. **C**: Voltammogram under non turnover (blue line) and turnover (orange line) with nitrate as substrate (Scan rate: 1 mV · s^{-1}).

Furthermore, we explored our biofilm-based system under autotrophic conditions regarding ammonium oxidation, and no appreciable shift in current density was detected when medium was spiked with ammonium (**Fig. 5 SI**).

The ability of PPB to uptake electrons from standard graphite electrode has been widely described [10,44]. Therefore, to study the cathodic processes that our consortium may catalyse, we used nitrate as a model reducible compound. In the presence of NO_3^-, the voltammogram showed an increase in the cathodic activity at potentials below 0 V. These voltammograms were like those previously reported[5]. These results suggested that the PPB consortium might be able to uptake electrons to reduce nitrate in which the Ef2 site would participate. The similarity of this voltammogram (**Fig.5C - Blue line**) with the one obtained in the ME-FBR_{IR} (**Fig. 4B**) suggests that the reduction peak observed could be related to some reduction reaction catalysed by the consortium. Therefore, a fluid-like electrode might also serve as electron donor for PPB allowing relevant applications such as nitrate reduction or bioelectrosynthesis.

Synergic effect between PPB and fluid-like electrodes

We operated both reactors, (**Fig. 1**) fed with brewery wastewater, to obtain steady state in regards of Total Organic Carbon (TOC) and Total Nitrogen (TN) removal rates.
Under OCP, no significant differences were observed in the TOC removal rate between both reactors. Therefore, the results indicated that just the presence of PPB did not result in a measurable enhancement in biodegradation in a ME-FBR without polarization.

Other authors have shown that the mere presence of the electrically conductive material accelerates the microbial metabolic processes without the need for external polarization[23,33,49]. This was confirmed by the results obtained in the ME-FBR_{DARK}, in which we observed that there were no significant differences regarding TOC removal in absence of polarization (OCP_{DARK}: 167.5 g TOC ·m^3·day^{-1}) and under different values of polarization (185.5 g ·m^3·day^{-1} at 0.2 V and 131.1 g TOC ·m^3·day^{-1} at 0.4 V) (**Fig. 6A**). Methane production and microbial community analysis (**Fig. 2**) suggest that, despite presenting similar biodegradation rates, the microbiological processes in both systems (OCP_{IR} and OCP_{DARK}) are diametrically opposed.

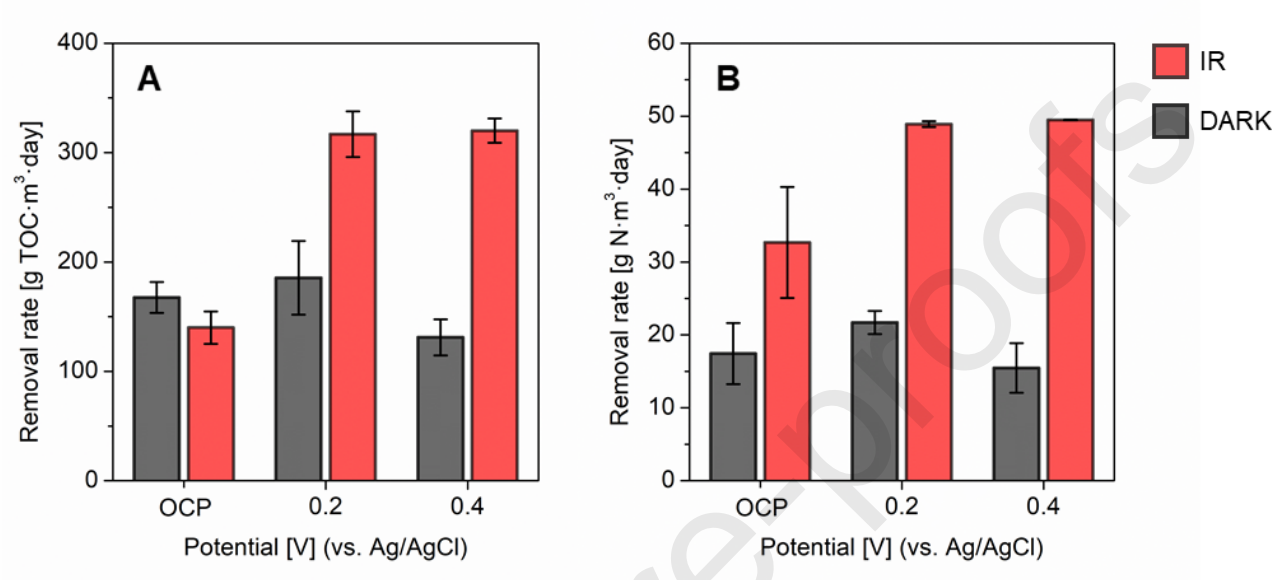


Figure 6. A: Total Organic Carbon (TOC) removal rate ± standard error under Open Circuit Potencial (OCP), 0.2 V and 0.4 V. B: Total Nitrogen (TN) removal rate ± standard error under Open Circuit Potencial (OCP), 0.2 V and 0.4 V. HRT=48 hours.

In contrast with a conventional ME-FBR, infrared illumination led ME-FBR_{IR} to a 2-fold increase in TOC removal rate under external polarization conditions at 0.2 V and 0.4 V (316.9 g ·m^3·day^{-1} and 320.1 g·m^3·day^{-1} respectively) compared with non-polarized conditions (OCP_{IR}: 140.0 g ·m^3·day^{-1}). The presence of electrically conductive material under OCP did not stimulate PPB in comparison with the electrode-free control (**Fig. 2 SI**), and electric current through an external circuit seem to be a strong requirement to enhance the metabolism of the PPBs. Furthermore, throughout the experiment, we did not detect concentrations higher than 4% of methane in the ME-FBR_{IR}. This indicates that infrared radiation, by promoting the growth of PPB in the reactor, prevents the activity of methanogenic bacteria. In summary, these results indicated that both IR radiation and external polarization must coexist to achieve a significant improvement in the TOC removal.

Regarding the nitrogen removal rates, ME-FBR_{IR} also outperformed the ME-FBR_{DARK} throughout the experiment (**Fig. 6B**). In dark conditions, we did not observe any significant effect of external polarization on TN removal. Under OCP conditions, ME-FBR_{IR} achieved rates of 32.7 g ·m^3·day^{-1}, slightly higher than those obtained in the ME-FBR_{DARK} (17.4 g ·m^3·day^{-1}). However, when we applied external polarization to the illuminated reactor (ME-FBR_{IR}) we observed an increase of close to 50% in the removal of TN as compared to the OCP_{IR}. The main nitrogen compound in the influent was ammonium, although low concentrations of nitrate and nitrite were detected. The ability of PPB to assimilate nitrogen as biomass[7,50] and our negative results for electrochemically-assisted ammonium oxidation suggested that assimilation is the main nitrogen removal process in the ME-FBR_{IR}.

Fluid-like electrode can modulate PPB biomass production

The use of PPB in wastewater treatment has been successful in nutrient and energy recovery through nutrient partitioning[51]. Biomass growth and yield are key in nutrient recovery so we have studied if electrochemistry and IR radiation can be used as tools to drive biomass production.

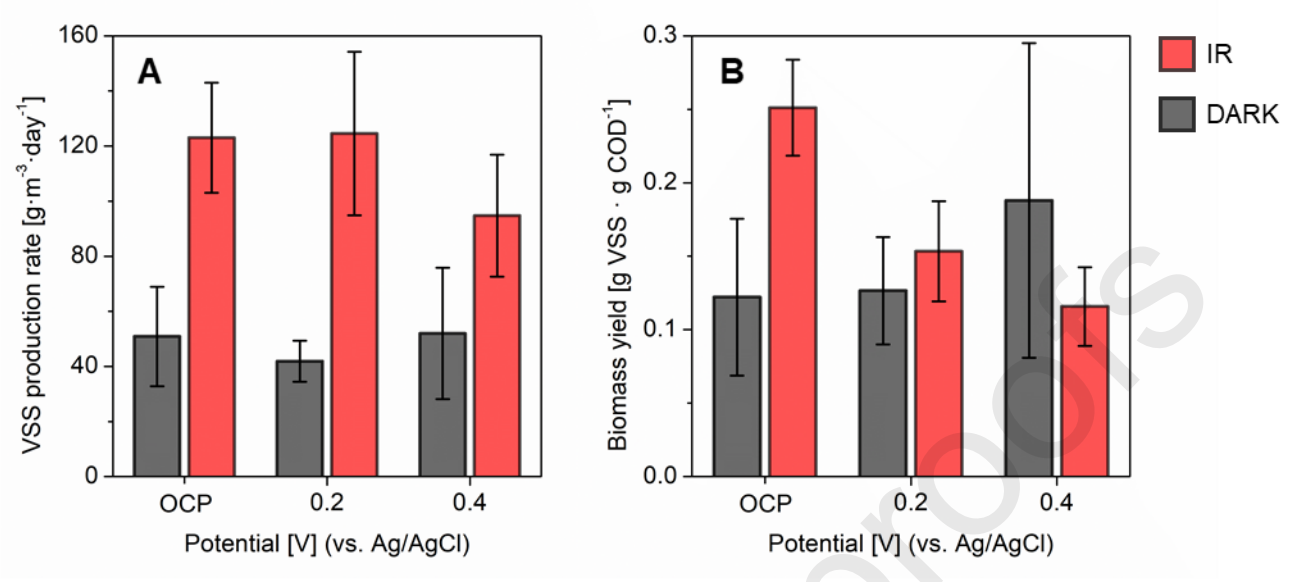


Figure 7. A: Volatile Suspended Solids (VSS) production rate ± standard error under Open Circuit Potencial (OCP), 0.2 V and 0.4 V. B: Biomass yield ± standard error under Open Circuit Potencial (OCP), 0.2 V and 0.4 V. HRT=48 hours.

The impact of electrode potential on biomass production is reported to be controversial since polarization can both enhance[52] or supress[53] cell growth. Thus, biomass production rates and biomass yields were examined in our ME-FBR to determine the role of electrode polarization. The results of both ME-FBR$_{DARK}$ and ME-FBR$_{IR}$ revealed that electrode potential does not have a significant effect on the biomass production rate (**Fig. 7A**). Regarding the effect of the IR illumination, we measured biomass production rates in the ME-FBR$_{IR}$ 3-fold higher than those from ME-FBR$_{DARK}$. The additional results (**Fig. 2 SI**) showed that the biomass production of a ME-FBR$_{IR}$ under OCP was not significantly different from an electrode-free reactor, which suggests that OCP$_{IR}$ behaves like a conventional PPB culture. The IR illumination promoted the growth of PPB in the ME-FBR$_{IR}$, which present assimilation rates as high as reported elsewhere[50] and supports why the biomass production rate was higher.

However, when we explored the conversion of organic pollutants to biomass (yield), we did observe an effect of polarization in the ME-FBR$_{IR}$. In the ME-FBR$_{DARK}$, we observed biomass yields like those described for other anaerobic sludge-based systems[54]. Biomass yield values between 0.1 and 0.2 gVSS · gCOD^{-1} were obtained, apart from the ME-FBR$_{IR}$ without external polarization (OCP$_{IR}$), in which the highest biomass yield was obtained (0.26 gVSS · gCOD^{-1}). This indicated that, despite the completely different metabolism of our electroactive microbial consortium, the biomass yield was similar to conventional anaerobic cultures. However, it is generally accepted that PPB consortia shows higher biomass yields than anaerobic sludge[55], which is consistent with the higher value observed in the OCP$_{IR}$. Carbon fixation as an electron sink has been described as one of the main reasons to justify why values of biomass yield is higher in PPB[41]. However, the PPB consortium biomass yield was reduced (**Fig. 7B**) under anodic polarization. It has been described that the presence of a polarized electrode (anode) can greatly affect the intracellular redox state (NAD$^+$/NADH ratio)[56]. Small variations in this ratio could trigger large metabolic effects[57]. Reduction in biomass yield may indicate that the Calvin cycle could be inhibited by the action of anodic polarization, favouring other electron utilizing pathways such as hydrogen production or nitrogen fixation which are less sensitive to the redox state of the cell[58] (**Fig. 8**).

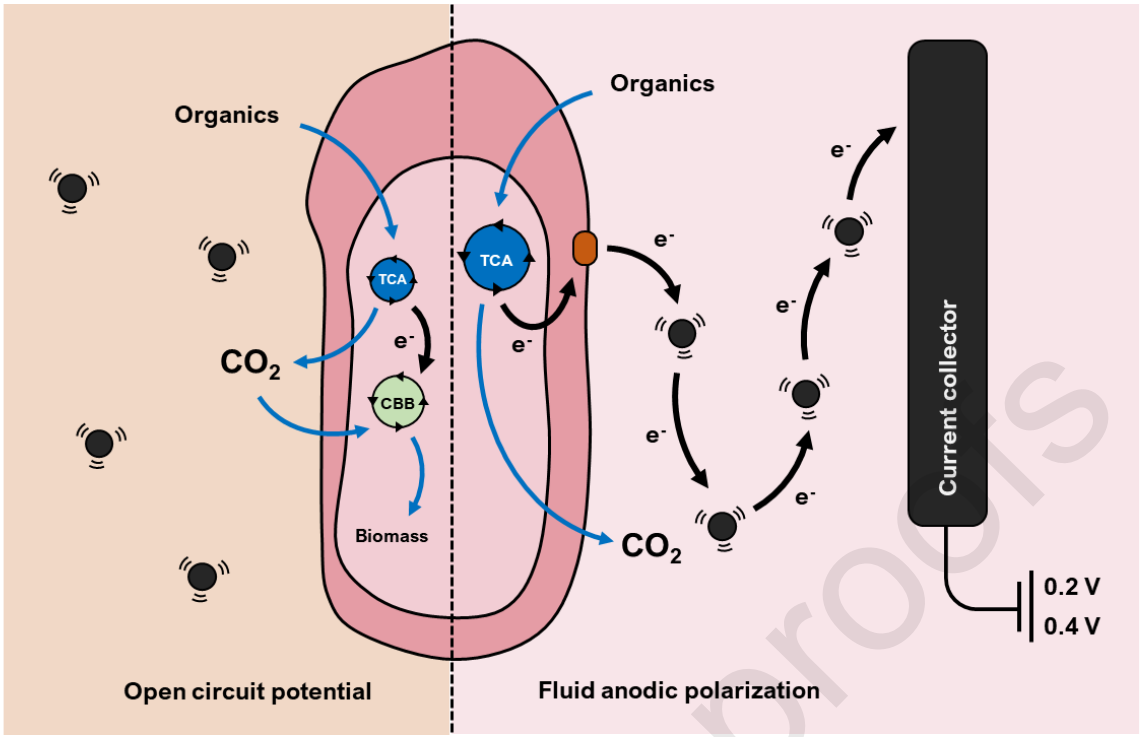
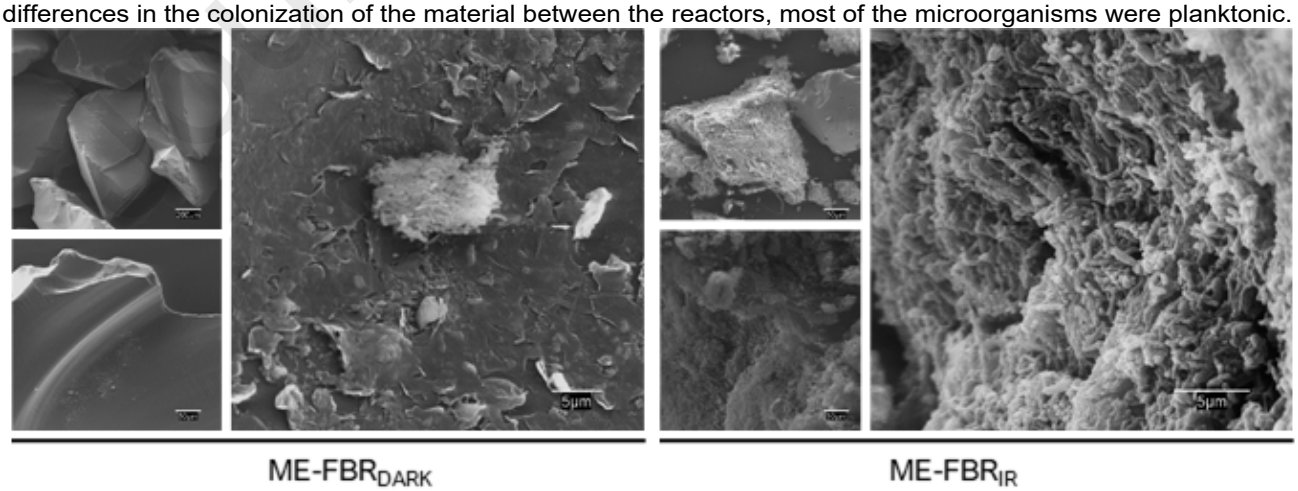


Figure 8. Conceptual model of the photoheterotrophic growth of Purple Phototrophic bacteria under open circuit conditions (left) and anodic polarization conditions in an electrochemical fluidized bed reactor. Under open circuit potential conditions, the PPBs use carbon fixation as an "electron sink" maximizing biomass production (left). Under fluid anodic polarization, PPBs use the electrode as an "electron sink" preventing CO_2 fixation (right). TCA stands for tricarboxylic acid cycle. CBB stands for Calvin–Benson–Bassham cycle.

PPB Planktonic growth predominates in infrared illuminated ME-FBR$_{IR}$

The biofilm formed on the surface of the fluidized bed particles was examined by scanning electron microscopy (SEM) after the polarization period. The low porosity of the vitreous carbon, together with the stress caused by the collision between the particles in fluidization, makes it difficult for the bed to colonize. In ME-FBR with vitreous carbon, planktonic interaction is favoured[21], which was consistent with the density of the biofilm observed in the reactors (Fig. 9). We found dispersed aggregates of microorganisms and extracellular substances attached to the surface, only in the areas most protected from collisions between fluidized particles. The predominant morphology in the ME-FBR$_{DARK}$ biofilm was short rods with a low presence of exopolymeric substances forming small aggregates. On the other hand, in ME-FBR$_{IR}$, we observed more colonization areas and a vast presence of EPS, in which rod-shape morphologies predominate. It should be noted that in some areas it was possible to observe rosette-like clusters, characteristic of mature cultures of the *Rhodopseudomonas* genus (SIIR6)[59]. Despite certain differences in the colonization of the material between the reactors, most of the microorganisms were planktonic.



Conclusions

Figure 9. Scanning electron microscopy (SEM) micrographs of the colonization on the particles of the fluidized reactors after two months of operation.

In this study, we have demonstrated that purple phototrophic bacteria can be grown planktonically and photoheterotrophically under the control of a fluid-like electrode. This novel method is remarkable by overcoming the limitation of the growth of electroactive PPB at a large scale with the conventional strategy of reliance on biofilm formation. We demonstrated for the first time that fluid-like electrodes can effectively electrobioremediate brewery wastewater using PPB, outperforming traditional electroactive non-photoheterotrophic processes. Additionally, our results indicated that IR illumination and external polarization must be used simultaneously to significantly improve TOC and nutrient removal. Furthermore, we observed that polarization and illumination play a fundamental role in microbiological and phenotypic selection, minimizing or eliminating methanogenic activity by improving the sustainability of the wastewater treatment process. Finally, we conclude that using fluid-like electrodes may accelerate the transition from the wastewater treatment model into the biorefinery model to maximize the recovery and reuse of water, carbon, and nitrogen.

Author Contributions

Carlos Manchon: conceptualization, investigation, writing original-draft and visualization. **Daniel Serna:** investigation. **Fernando Muniesa-Merino:** conceptualization, investigation and writing review & editing. **Yeray Asensio:** conceptualization, investigation and writing review & editing. **Colin Wardman:** writing review & editing**. Abraham Esteve-Núñez:** investigation, writing review & editing, project administration and funding acquisition.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was supported by the Spanish Ministry of Science, Innovation, and Universities—State Research Agency (AEI) and the European Regional Development Fund (ERDF) through the project MET-FLUID - Microbial electrochemical reactors based on fluid-like electrodes: a new biotech platform for performing environmental applications. Ref. RTI2018-101974-B-C-21 (MCIU/AEI/FEDER, UE). In addition, this work was also supported by Madrid Regional Government through the project REMTAVARES. Ref: P2018/EMT-4341. Carlos Manchon was funded by the Industrial Ph.D. fellowship program from the Regional Government of Madrid: IND2020/AMB-17843.

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Fluid-like electrodes and Purple Phototrophic Bacteria: bridging the gap in wastewater biorefineries

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HIGHLIGHTS

- **A Fluid-like electrode can accept electrons from the metabolism of planktonic PPB**
- **A Fluid-like anode enhances biodegradation rate (2-fold)**
- ***Geobacter* genus was the electroactive bacteria outcompeting under dark conditions**
- **Electroactive PPB outcompeted others under IR illumination and electrostimulation**
- **Fluid-like bed bioreactors are useful in recovering nutrients as PPB biomass**

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: