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MICROBIAL ELECTROLYSIS CELL: HYDROGEN PRODUCTION USING MICROBIAL CONSORTIA FROM ROMANIAN WATERS

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The present study aims to provide additional insight into the bioelectrochemical processes that drive biohydrogen production by microorganisms living in aqueous ecosystems. To this end, we have obtained water samples from three locations in Romania (the Black Sea, Lake Siutghiol and the River Sabar), and employed them in the cathodic chamber of a Microbial Electrolysis Cell (MEC) run at a negative polarization of 1,100mV vs. Ag|AgCl. The microbial species present in the water samples employed in the MEC proved capable of driving biohydrogen production through electrolysis without the need of mediators, reaching a maximum efficiency of 57% in biohydrogen production using the marine waters sample. Microbial activity also led to the reduction of nitrates present in the wastewater substrate; this may spell promising developments in wastewater treatment coupled with biohydrogen production.

Keywords: Microbial Electrolytic Cells, Biohydrogen, Wastewater treatment

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1. Introduction

Hydrogen serves as an excellent energy carrier in sustainable economic models based exclusively on renewable and alternative energy sources [1, 2], collectively branded as "Hydrogen Economy", with hydrogen-powered Fuel Cells (FCs) set at the technological foundation of the whole endeavor [3, 4]. Hydrogen production relies on: thermochemical processes (i.e. steam reforming) [5, 6], electrochemical processes (i.e. water electrolysis and photo-electrochemical water splitting) [7], or biological processes (i.e. biohydrogen generation) [8]. In the last decade, biohydrogen research has focused on: wastewater photolysis using green algae, anaerobic digestion of organic substrates by dark fermentation during the acidogenic phase, water-gas shift using photo-fermentation [7], bacterial fermentation of carbohydrates (e.g. glucose) [9], and bioelectrohydrogenesis [10]. The latter consists of an electrolytic process that transforms biodegradable organic substrates into biohydrogen by employing modified Microbial Fuel Cells (MFCs), thus termed Microbial Electrolysis Cells (MECs).

The first MEC model (MEC1) is built around an MFC architecture employing negative polarization at the anoxic cathode; protons generated during the microbial catabolic phase become reduced at the cathode under low potential supplied by an external electromotive force [11-17]. MEC1 has the distinct advantage over fermentation methods of reaching a higher biohydrogen yield, and over traditional water electrolysis of running at greater energy efficiencies, as the applied negative polarization is lower than the potentials required by electrolysis [18-21]. The second model (MEC2) applies negative polarization on microbial biofilms formed around the electrode in the anodic chamber; protons become reduced directly by the microorganisms.

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Key elements of MEC architecture that have instigated research interest are electrodes and catalysts, with efforts focusing on graphite vs. Platinum (high overpotential vs. high cost and catalyst poisoning) [22, 23]. Other areas of interest focus on investigating different types of biocatalytic microorganisms, biofilm formation, electron transfer mechanisms and redox molecules (e.g. membrane-bound cytochrome hemeproteins) [23]. Various types of bacteria (e.g. *Clostridium butyricum*, *Clostridium perfringens*, *Enterobacter aerogenes*, *Escherichia coli*, *Geobacter sulfurreducens*) are capable of accepting electrons and of generating hydrogen under anaerobic conditions; the most popular hydrogen-producing microorganisms are *C. butyricum* and *E. coli*, facultative anaerobes capable of fermenting both glucose and lactose [24].

Recent studies on MECs focus on the primary biochemical mechanisms of the microbial electron uptake at the cathode and on biohydrogen production mediated by the presence of inter-membranal enzymes (e.g. c-type cytochromes and hydrogenases) [25]. Less attention has been paid to biocompatibility and bioaffinity issues, and to biohydrogen production under direct application of negative polarization to biofilms in the anolyte chamber. When dealing with large populations of wastewater microorganisms, we expect to observe biohydrogen production with the simultaneous reduction of nitrate species in the substrate, provided there are nitrate-reducing bacteria in the microbial population or nitrates serve as terminal electron acceptors. For this reason, we have conducted a series of experiments employing a bi-chamber MFC with negative polarization directly applied on biofilms (i.e. a MEC2), using water samples collected from three locations in Romania: the Black Sea (high salinity waters), Lake Siutghiol (freshwater depository near the Black Sea coastal area) and the River Sabar (near Bucharest, with considerable wastewater influents from riparian rural communities). The experiments focused on investigating the MFC-to-MEC transition stage while considering critical polarization thresholds, and on evaluating biohydrogen production and nitrate removal capacities.

2. Theoretical background

2.1 Microbial Fuel Cell

Figure 1 portrays the MFC operation principle: microbial consortia catabolize the organic substrate, forming biofilms and transferring excess electrons (exoelectrons) to the anode. Electrodes are constructed using conductive anticorrosive materials (e.g. graphite rods, mesh or brushes) with high specific surface area; membranes (PEM) employ proton-conducting materials (e.g. Nafion); the anodic chamber contains a biotic solution with microbial consortia, while the cathodic chamber contains an abiotic medium (buffer solution or mineral medium) [16].

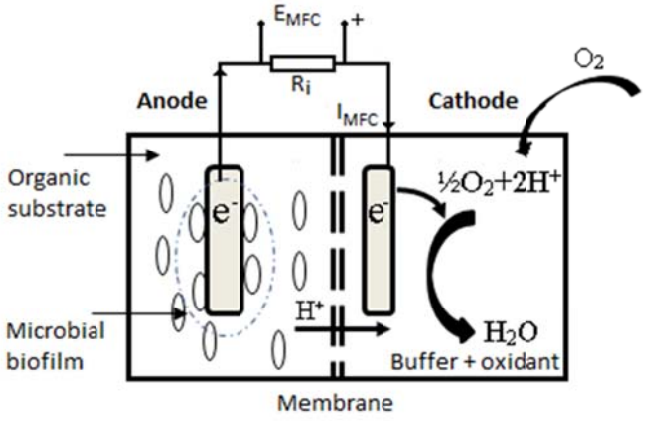

Fig. 1: MFC operation principle. Microbial consortia catabolize the organic substrate, forming biofilms and transferring exoelectrons to the anode; protons migrate through the PEM to combine with oxygen, forming water. I_{MFC} is the current generated by the MFC, R_i the equivalent internal resistance and E_{MFC} the generated potential.

A key issue in biofilm development - and thus exoelectron transfer - is the bioaffinity between the electrode material and the microorganisms (a biofilm-encrusted anode/cathode is commonly referred to as a bioanode/biocathode). Oxidation of the organic substratum releases protons, which migrate through the proton-exchange membrane into the cathode chamber, where they recombine with atmospheric oxygen to form water. The equivalent circuit consists of an EMF gradient (E_{MFC}) providing an open-circuit voltage (V_{OC}) over the internal resistance (R_i) of the total circuit elements. Microbes consume a fraction of the electrons produced by substrate oxidation (F_s) to provide energy required for cell growth; surplus electrons are transferred to the outer cell membrane ($F_{e\text{-}cell}$), where they are used for energy production (F_x) – excess electrons are expelled to the anode as exoelectrons (F_{exo}). The overall equilibrium holds as:

$$F_s > F_{e-cell} = F_x + F_{exo} \quad (1)$$

The chemical composition of the organic fraction in wastewater varies according to its origin. As a rule of thumb, often evoked in wastewater treatment, the organic fraction can be represented by a generic compound ($C_{18}H_{19}O_9N$) with a mean molar mass of ~393g [26, 27]. When oxidized by microbes (without nitrification), the end products are carbon dioxide, water and ammonia according to the formula:

$$C_{18}H_{19}O_9N + 17.5O_2 + H^+ \rightarrow 18CO_2 + 8H_2O + NH_4^+ \quad (2)$$

The above reaction yields a BOD value of ~1.42kg O_2/kg of organic matter. To estimate the energy yield of a typical MFC, we need to account for the Gibbs free energy (ΔG^0, in joules per electron equivalent, under standard biological conditions of: p=1atm, T=25^{0}C, pH=7) in the following half-reactions [26, 27]:

$$\frac{1}{70}C_{18}H_{19}O_9N + \frac{28}{70}H_2O \rightarrow \frac{17}{70}CO_2 + \frac{1}{70}HCO_3^- + \frac{1}{70}NH_4^+ + H^+ + e^- \quad (3)$$

$$\Delta G_{aq}^0 = +32kj/eeq, E_{0a} \approx -0.33V \quad (4)$$

, where the oxidation potential E_0 is calculated according to $E_0 = -\Delta G^0/F$ (F stands for Faraday's constant). The reactions in the cathode chamber yield:

$$\begin{aligned} &\frac{1}{2}O_2 + H_2 \xrightarrow{\Delta G^0 = -237.34\ kJ/mole} H_2O(l) \Leftrightarrow \\ &\frac{1}{4}O_2 + H^+ + e^- \xrightarrow{\Delta G^0 = -118.67\ kJ/eeq} \frac{1}{2}H_2O;\ E_{0c} = 1.23V \end{aligned} \quad (5)$$

Correcting for neutral pH:

$$E_{0c}^{'} = E_{0c} - \frac{RT}{nF}\ln\frac{[H_2O]^{1/2}}{[pO_2]^{\frac{1}{4}}[H^+]} \cong 0.804V \quad (6)$$

, with the reduction potential being calculated for an air-bubbling chamber at 1atm with an oxygen partial pressure [pO_2]=0.2atm and [H^+]=10^{-7}M.

The electromotive force per electron equivalent is:

$$E_{MFC} = E_{0c}^{'} - E_{0a}^{'} \cong 0.804 - (-0.33) \cong 1.134V \quad (1)$$

The above value stands for the theoretical potential reached by an MFC, when the organic substratum becomes fully oxidized and the transfer fraction F_s of electrons to the bacterial cell reaches 100%. In non-theoretical cases, E_{MFC} ranges from 400 to 700mV for monocultures (the typical values differ for microbial consortia) [28]. For our water samples, maximal V_{OC} range from 300 to 400mV due to the biotic solution employed as organic substratum in our MFC and the varying composition of the microbial consortia (Table 1 in the Results section).

2.2 Microbial Electrolysis Cell

In the first model of MEC architecture, hydrogen is produced via bioelectrohydrogenesis, a procedure that requires a negative polarization over the cathode and anoxic operating conditions in the cathodic chamber (Fig. 2). Protons released from the bioanode migrate through the PEM to become reduced in anoxic conditions:

$$H^+ + e^- \rightarrow \tfrac{1}{2}H_2;\ E_0 = 0V \text{(standard conditions)} \qquad (8)$$

Adjusting for neutral pH:

$$E^{'} = 0 - \frac{RT}{nF}\ln\frac{[H_2]^{1/2}}{[H^+]} = -\frac{RT}{nF}\ln\frac{1}{[10^{-7}M]} \cong -0.414V \qquad (9)$$

The direct negative polarization , applied via an external source (E_{ext}) over the cathode, must be $|E_{ext}| > E_{MFC} + E' \approx 0.7V$, according to the theoretical redox potential value calculated in the previous section [11, 16].

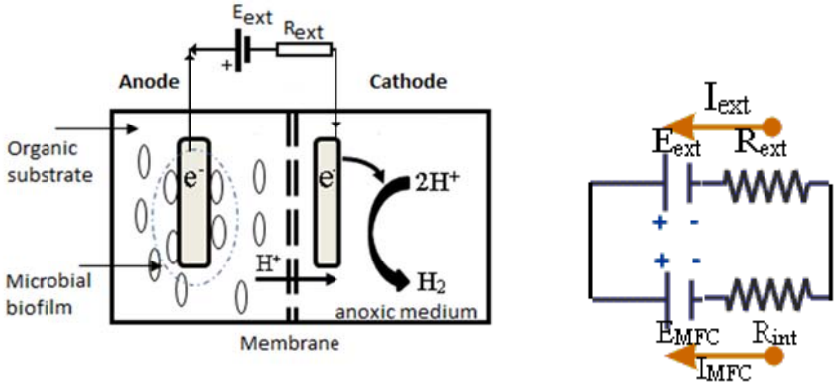

Fig. 2: *MEC1 configuration, with negative polarization on the biocathode provided by an external source (E_{ext}); protons and excess electrons combine in the anoxic cathode to release hydrogen gas. R_{ext} is the equivalent resistance of the external circuit; internal resistance R_i and generated potential E_{MFC} are shown in the equivalent circuit.*

The second model of MEC architecture requires the negative polarization of the bioanode, in order to transfer electrons from the external electrical source to the biofilm (Fig. 3). Direct biohydrogen generation takes place during the acidogenic phase of the anaerobic digestion of the organic substrate. The negative polarization of the bioanode directs an excess of electrons to the biofilm, forcing the MEC to function in reversal (i.e. the bioanode becomes the biocathode); in this case, the external electromotive force (E_{ext}) must be higher than the open circuit voltage generated by the MFC: $|E_{ext}| > E_{MFC}$ [16].

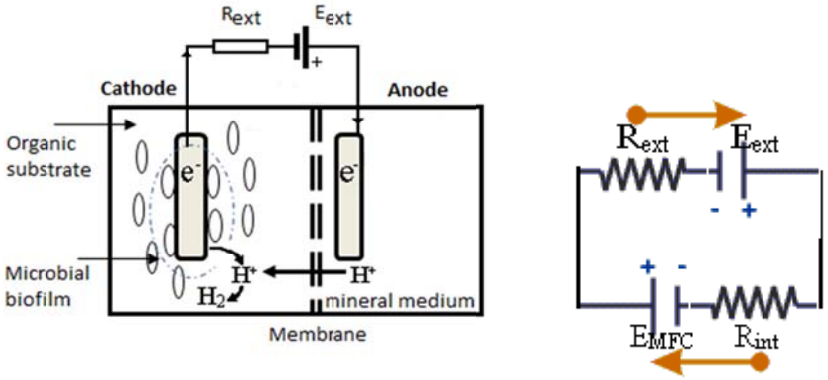

Fig. 3: *MEC2 configuration, with negative polarization on the biocathode provided by an external source (E_{ext}), after electrode polarization reversal; protons from the mineral medium migrate through the PEM and, together with protons released through the oxidation of the organic substratum, combine with electrons inside the bacterial cells to produce hydrogen in the cathodic chamber. R_{ext} is the equivalent resistance of the external circuit; internal resistance R_i and generated potential E_{MFC} are shown in the equivalent circuit.*

The mechanism for hydrogen generation in MEC2 is quite different than in MEC1, which is based on hydrogen reduction in an anoxic medium. By negatively polarizing the biofilm in MEC2, external electrons are supplied to the cellular (periplasmic) membranes of the microbes, where protons are reduced by specific enzymes: hydrogenases and nitrogenases (responsible for reducing nitrates to nitrites) biased by the cytochrome complex, an essential component of the electron transfer chain [31, 32]; if the hydrogen-producing metabolic pathway cannot be accessed, then the process continues with other available electron acceptors (e.g. nitrates).

Few naturally occurring microorganisms carry the above set of enzymes: green algae, cyanobacteria and dark fermentative microbes [33-35]. Microbial consortia exhibit more complex electron transfer mechanisms, often with non-synergetic effects pertaining to hydrogen production or pollutant removal [36].

At the electrode-biofilm interface, electron transfer can occur either directly, when the biofilm is in direct physical contact with the electrode, or indirectly, when redox reactions are carried out by chemical mediators [23]. In either case, microbes release redox-active compounds by metabolizing organic substrates to transfer electrons to and from the electrodes.

3. Materials and Methods

3.1 Materials

The following materials were used in the assembly and operation of the MEC:

Water: Deionized water (DI), distilled water (DW) and samples from the aforementioned locations, collected and stored in sterilized containers.

Standard abiotic solution: mineral medium with standardized composition: NH_4Cl at 0.51g/L, $MgCl_2 \times 6H_2O$ at 0.102g/L, K_2HPO_4 at 0.4g/L and $CaCl_2 \times 2H_2O$ at 0.05g/L. All chemicals were of analytical grade and used as received.

Anodic chamber: containing the anolyte solution with the standard abiotic medium.

Cathodic chamber: containing solutions made of a reference abiotic solution and each of the three water samples, respectively.

Anodic electrode: SIGRADUR® glassy carbon (HTW Hochtemperatur-Werkstoffe GmbH, Germany), 2cm^2 surface area.

Cathodic electrode: graphite rod (Sigma Aldrich®), 4.14cm^2 surface area. The graphite rod was activated before use as follows: 1) soaked for 1h in HCl (12M), washed in DW, then soaked for 24h in HCl (1M) and washed again; 2) soaked for 24h in NaOH (1M), washed in DW, then soaked for 24h in HCl (1M) and washed; 3) soaked for 24h in NaOH (1M), washed and kept in DW before use.

Proton-exchange membrane: Nafion 117, DuPont. PEM was activated by boiling in H_2O_2 (3% v/v) for 2h, then in H_2SO_4 (0.5M) for 2h and finally in DI water for 2h and stored in DI water before use.

3.2 Experimental setup

The MEC2 setup used in our experiments consisted of two airtight glass bottles (250ml) separated by a 3cm^2 (cross-section area) PEM. The anodic chamber contained 150ml of the abiotic solution; the cathodic chamber contained 160ml of the biotic solution-water sample mixture, and housed the graphite rod electrode and an Ag|AgCl reference electrode at +199mV vs. SHE. Before use, each chamber was purged with a gas mixture of N_2/CO_2 (70/30% v/v) for 30min (10min in the liquid phase and 20min in the gas phase) to remove oxygen/hydrogen residues; all solutions were adjusted to neutral pH. The system was maintained at 35^{0}C in a water bath under stirring to ensure that mass transfer would not affect current generation.

3.3 Analytical techniques

The following methods and instrumentation were used throughout our analysis:
Electrochemical Impedance Spectroscopy & Cyclic Voltammetry: VoltaLab® 40 (PGZ301 & VoltaMaster 4) analytical radiometer. The scanning range for Cyclic Voltammetry was set at -1200 to 500mV vs. Ag|AgCl at a scan rate of 10mV/s, to measure microbial redox activities.
Chronoamperometry: Electrical current time series were recorded at a time interval of 30s for 8h at a fixed polarization potential of -1100mV vs. Ag|AgCl, to measure hydrogen kinetics and coulombic efficiencies (charge accumulation in μeqQ). All hydrogen gas produced during electrolysis was collected from the cathode headspace using a sample lock Hamilton syringe (500µl) and then transferred to the gas chromatograph.
Gas Chromatography: Varian® 3400 GC, stainless steel columns with molecular sieves, *He* gas carrier at 18ml/min, oven temperature at 180^{0}C, thermal conductivity detector at 200^{0}C. Hydrogen content was measured using the Residual Gas Analyzer (detection limit at 0.02ppm). Sulfates, nitrates and chlorides were measured by Ionic Exchange Chromatography using column and pre-column A522 at 4mm; a Na_2CO_3 (3.5mM) and $NaHCO_3$ (1mM) solution was used as eluent at a flow rate of 1.2ml/min. The samples were filtered through a Millipore 0.2µm and diluted with DI.

4. Results and discussion

4.1 Cyclic voltammetry

The basic mechanism in MFC operation lies in the transfer of electrons produced by microbial respiration to an electrode, instead of a terminal electron acceptor. Microbial consortia form biofilms on the surface of the electrode and catabolize the organic substratum, transferring exoelectrons collected by the electrode to an external circuit, thus doing work and generating a potential difference (V_{OC}) between the electrodes of the MFC. Exoelectrons are stored as accumulated charge in Double Layer Capacitance (C_{DL}) formed between the biofilm and the electrode; this can be estimated by measuring the average between anodic (I_a) and cathodic (I_c) current densities at 0V vs. SHE (-0.2V vs. Ag|AgCl) by cyclic voltammetry, according to the current/voltage relationship [16]:

$$\bar{I}(t) = \tfrac{1}{2}(I_a - I_c) = C_{DL}\frac{dV}{dt} \qquad (10)$$

, where dV/dt is the scan rate (V/s). Table 1 shows V_{OC} and total accumulated charge values (Q_{DL}) of the C_{DL} for the three water samples (capacitance of mineral medium set constant at 44mF/cm^2).

Table 1: Open circuit voltage (V_{OC}), double layer specific capacitance (C_{DL}) and accumulated charge (Q_{DL}) measurements for all samples using graphite rod electrodes (in parenthesis under V_{OC}, the respective values for carbon paper electrodes); under $\#e^-$, the electron densities and under M_{bio}, the total biofilm mass for each sample.

Sample	$V_{OC}(mV)$	$C_{DL}(mF/cm^2)$	$\bar{Q}_{DL}(C)$	$\#e^-(eq/\mu mole)$	$M_{bio}(\mu g)$
Black Sea	428.0 (364.2)	350 to 400	0.150 to 0.750	7.80	43.8
River Sabar	322.5 (320.8)	170 to 200	0.064 to 0.280	2.90	16.3
Lake Siutghiol	311.0 (289.1)	40	0.012 to 0.053	0.55	3.1

Cyclic voltammetry was used to establish the electron transfer mechanism and to estimate the microbial electrocatalytic activity at the graphite electrodes. Figure 4 shows typical voltammograms of the biofilms, recorded at a scan rate of 10mV/s after 48h of continuous electrode polarization at -1100mV vs. Ag|AgCl. For comparison, the voltammogram of an identical abiotic electrode (i.e. blank sample) in anaerobic conditions has been included; as expected, voltammetry of the abiotic electrode has not revealed any occurrence of significant redox processes in the window +200 to -1200mV vs. Ag|AgCl).

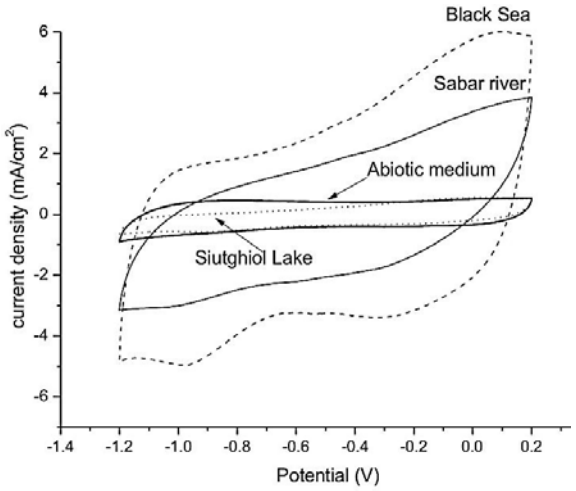

Fig. 4: *Cyclic voltammetry for water samples and abiotic medium, at a scan rate of 10mV/s. CVs are recorded after polarization at -1100mV vs. Ag|AgCl for 48h.*

In the presence of the microbial biofilms, the cathodic current corresponding to hydrogen reduction ranged from –600mV to -1000mV for the Black Sea water sample. The voltage required for hydrogen production stayed close to previously reported ones: around -600mV vs. Ag|AgCl using Pt-based cathodes [37] and -950mV using stainless steel and specific microbial species [38]. Observed values of current densities for the Black Sea sample were higher than other reports – in our cases, we also observed large DL capacitance and low biomass density of biofilms. During the anodic sweep of the voltammetry, we detected no anodic peak corresponding to H_2 oxidation; this is indicative of a substantial catalytic bias of the enzymes, which seem to be more active in hydrogen-production phase, when terminal electron acceptors (acting as a sink for the electrons produced by H_2 oxidation) are limited. The waters from Sabar River and Siutghiol Lake showed very low hydrogen productivities, the microbial consortia being either very low in concentration or not appropriate for bioelectrolysis. The voltammograms also displayed smooth slopes, associated with the gradual activation of enzymes in contact with the electrode under polarization - the possibility of activating (or deactivating) hydrogenases attached onto a carbon-based electrode by electrochemical control has been reported in past works [39]. Continuously increasing the anodic potential over -300mV giving a very low cathodic peak at -250 to -300mV is compatible with c-type cytochromal activity. By comparison, the Black Sea microbial community displayed a high

capacity to accept electrons and a higher charge accumulation during bioelectrolysis - the bioelectrochemical activities of the microbial communities are also closely influenced by the level of organic compounds (e.g. sulfates, nitrites, chlorides) that can poison their oxidative metabolism.

4.2 Chronoamperometric analysis

Electric charge accumulation was measured in μeqQ's from current-time polarization curves. Hydrogen concentrations have been evaluated from gas chromatographic measurements and the cumulative equivalents for hydrogen production (μeqH_2) have been measured, taking into account a molar conversion factor of 2μeq/μmol; thus, hydrogen production efficiency was calculated as:

$$E_{H_2}\% = (\mu eqH_2/\mu eqQ)\ x\ 100\% \qquad (10)$$

Hydrogen production efficiencies calculated for the water samples are summarized in Table 2 and Figure 5. For each sample (except the blank), charge accumulation and hydrogen production increased over time, as a function of electrolyte ionic composition and the associated kinetics through the cationic membrane. In the Black Sea sample, these reach their maximal values; microbial biofilm density and activity were also much higher than in the other samples, in agreement with their respective efficiencies, indicating that the microbial consortia display different capacities for extracellular electron transfer at the electrodes during hydrogen generation. However, hydrogen production efficiencies displayed a different trend: in the Black Sea sample, efficiency kept rising even after the 8h mark, when it reached a value of ~57%; in the River Sabar and Lake Siutghiol samples, efficiencies reached low peaks (at ~25% and ~5% respectively) at the 4h mark and kept diminishing gradually until they almost zeroed at 8h. Thus, the microbial consortia from River Sabar and Lake Siutghiol do not offer themselves for bioelectrolysis: their bioaffinities to the graphite electrode are comparatively low – most probably another kind of nanostructured material is needed for the electrode to improve their bioactivities.

Table 2: Hydrogen productivities and accumulated charges under a polarization of -0.110V vs. Ag|AgCl.

Time	Abiotic medium		Black Sea		River Sabar		Lake Siutghiol	
h	*μeqQ*	*μeqH₂*	*μeqQ*	*μeqH₂*	*μeqQ*	*μeqH₂*	*μeqQ*	*μeqH₂*
2	9.70	0.00	13.80	0.00	2.64	0.00	3.65	0.00
4	14.55	0.00	24.25	8.88	8.95	2.20	3.88	0.19
6	18.65	0.00	33.20	16.56	13.80	1.97	11.19	0.29
8	25.74	0.00	35.81	20.65	19.77	1.03	14.55	0.32

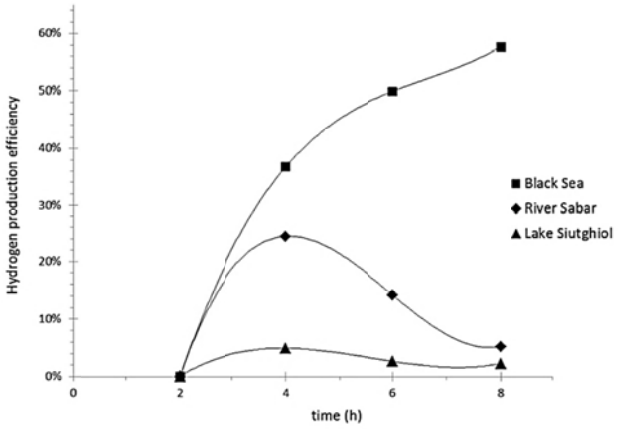

Fig. 5: *Hydrogen production efficiency as a function of time*.

4.3 Nitrate residues

Table 3 shows the microbial capacity to reduce nitrate compounds in the cathodic chamber, while producing hydrogen during bioelectrolysis. All samples showed a decrease in nitrate concentration; in the case of the Black Sea sample, nitrate was fully reduced - this does not necessarily imply a greater reduction capacity for the respective consortia, as the starting value of nitrates concentration in that configuration was small in the first place. Since the experiments have been repeated a number of times for reproducibility, every time yielding the same results, the correlation between nitrate reduction and bioelectrolysis can be readily assumed as a fact. However, establishing the exact nature of the underlying phenomena to investigate causation needs to be examined by more directed experiments, which go beyond the scope of the present work.

Table 3: Nitrate residues in the cathode after bioelectrolysis for all samples.

Sample	Initial conc. (*mmol*)	Final conc. (*mmol*)	Percentage decrease (*%*)
Black Sea	0.69	0.00	100.00
River Sabar	7.82	5.44	30.43
Lake Siutghiol	3.54	1.36	61.58

5. Conclusions

MECs provide an effective method for hydrogen recovery from different waters (e.g. wastewaters, aqueous ecosystems) that contain microbial consortia which commonly employ multi-enzymatic metabolic pathways; consortia of such synergistic organization obtain the capacity for longevity during the bioelectrolysis process and the capability to utilize a wide selection of organic substrata.

In our experiments, we have employed MEC2 configurations (-900mV vs. SHE negative polarization applied on the biocathodes), using graphite electrodes and biological loads obtained from water samples that were collected from three locations in Romania: the Black Sea, Lake Siutghiol and the River Sabar. The microbial consortia present in the biological loads shown varying degrees of synergy, which enabled intraspecies and interspecies electron transfer, and formed biofilms with different bioaffinities to the electrode material. These factors drastically affected biohydrogen production efficiencies: the MEC system loaded with the Black Sea sample (marine waters) has the highest efficiency, reaching the value of 57.7% after 8 hours of

bioelectrolysis (local maximum, as the process had not reached termination even after the 8-hour interval); the other samples have much lower efficiencies, reaching their peak values after 4 hours, which gradually diminished towards termination after 8 hours - the lowest efficiency of 2.2% was obtained from the Lake Siutghiol sample (freshwaters).

As a secondary objective to our experiments, we have carefully monitored nitrate residues in the cathodic chambers of the MECs, before and after hydrogen kinetics measurements - nitrate acts as an important nutrient in aqueous ecosystems and high nitrate concentrations signal the onset of eutrophication outbreaks that pose a severe environmental hazard; thus, monitoring nitrate residues offers insights as to the compatibility of biohydrogen production using MECs in wastewater treatment. Nitrate concentrations diminished in all three of our samples during bioelectrolysis after an 8-hour interval. The exact mechanism of this phenomenon has not been investigated further – it nevertheless provides a milestone into further research concerning bioelectrolysis applications in wastewater treatment.

Acknowledgements

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