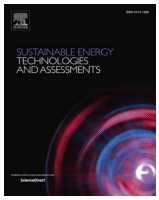
Sustainable Energy Technologies and Assessments 54 (2022) 102805

Contents lists available at ScienceDirect

Sustainable Energy Technologies and Assessments

journal homepage: www.elsevier.com/locate/seta



The degradation of glyphosate is enhanced in a microbial fuel cell: Electrochemical performance, degradation efficiency, and analysis of the anodic microbial community

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ARTICLE INFO

Keywords:
Microbial electrochemical systems
Glyphosate
Microbial community
Biodegradation

ABSTRACT

Glyphosate, one of the most used herbicides worldwide, is known as an aquatic contaminant of concern, and has been identified as presenting adverse impacts in agroecosystems, due to a somewhat limited natural chemical and biological degradation in the environment. In this study, we investigated the degradation of glyphosate in microbial electrochemical systems (MESs), and compared the performance and the microbial composition of enriched anodic biofilms with those shown by native microbial communities. The reduction of glyphosate content observed in MESs (approx. 70 %) was much higher than in non-electroactive microbial cultures (approx. 49 %). The analysis of the microbial communities by 16S amplicon sequencing revealed a significant difference between the microbial community composition of MESs anodic biofilms and non-electroactive enriched communities. The anodic biofilms were dominated by *Rhodococcus* (51.26 %), *Pseudomonas* (10.77 %), and *Geobacter* (8.67 %) while in non-MESs cultures, methanogens including *Methanobrevibacter* (51.18 %), and *Methanobacterium* (10.32 %), were the dominant genera. The present study suggested that MESs could be considered as a promising system for complete degradation of glyphosate from waters polluted by this herbicide.

Introduction

Pesticides are among the most important contaminants of aquatic environments and present associated health risks to humans and animals, affecting diverse different ecosystems [10]. Glyphosate (N-((phosphonomethyl) glycine, PMG) is a broad-spectrum herbicide, widely used for agricultural applications in more than 140 countries [31]. The global use of glyphosate in 2014 reached 826 million kilograms, a 12-fold increase compared to global usage in 1995 [7], and it is expected to reach 920 million kilograms by 2025 [28].

The intensive use of glyphosate in the last decades has resulted in the contamination of surface and groundwaters through drainage, wind, soil erosion, and runoff [40]. The ecotoxicity of glyphosate has been reported to cause destruction in the balance of aquatic ecosystems [32,35,43]. Glyphosate concentrations in various aquatic environments have been reported to be in the range from ng. l^{-1} to 2.8 mg. l^{-1} [27]. For instance, the highest residual concentration of glyphosate in surface waters reached up to 700 μg.l^{-1} in Argentina [34], 430 μg. l^{-1} in the USA [29], and 165 μg. l^{-1} in Europe (Villeneuve et al., 2011).

The presence of glyphosate in the environment is of concern, as it can adversely affect the health and safety of plants, animals, and humans [37]. Several studies have highlighted the effects of the herbicide in humans. Glyphosate is an endocrine disruptor in humans and might be carcinogenic in high concentrations [5]. Furthermore, it has been reported that glyphosate can affect the composition of the gut microbiota and subsequently the central nervous system [39]. In a recent study, the glyphosate concentrations in urine samples collected from 6848 French participants between 2018 and 2020 was analysed. The results revealed that more than 99 % of the urine samples contained quantifiable levels of glyphosate [18]. That study revealed that widespread contamination of glyphosate in France, an industrialized country, was mainly via ingestion, as individuals who consumed organic food and drank filtered water had lower levels of glyphosate in their urine. In terms of the effect on microorganisms, glyphosate exposure changed the composition of gut microbiota in rats, which was unexpectedly correlated negatively with male reproductive toxicity [26]. The extensive collection of experimental data demonstrates the need for complete elimination of glyphosate from the environment.

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https://doi.org/10.1016/j.seta.2022.102805
Received 18 March 2022; Received in revised form 5 September 2022; Accepted 27 September 2022
Available online 12 October 2022

Several approaches can be used to treat glyphosate contaminated waters. Conventional methods including adsorption and biological treatments have been applied to treat water polluted with glyphosate [45]. Also, advanced oxidation processes such as photocatalysis and electrochemical oxidation have been studied as alternative technologies for the removal of a variety of organic pollutants, including glyphosate [12], and integration and combination with biological treatments have been proposed as promising approaches for the efficient removal of a range of recalcitrant organic pollutants from wastewaters [50]. Among them, the use of Microbial Electrochemical Systems (MESs), combining biological degradation with electrochemical oxidation, is considered as a powerful emerging technology. MESs have been studied to remove a wide range of organic pollutants while recovering energy and valuable chemicals [36,46]. Due to the synergy between electrochemical processes and biological degradation, MESs could support higher removal of recalcitrant pollutants compared to biological degradation alone [33]. While the microbial degradation of glyphosate has been studied in some detail [24], the use of microbial electrochemical technology for elimination of this pollutant has not been attempted so far.

In this work, we studied the degradation of glyphosate in microbial fuel cells (MFCs). We inoculated a natural microbial community in the anode of a glyphosate-fed MFC and assessed the efficiency of the acclimatised biofilm to degrade glyphosate, monitoring the electrochemical performance of the systems and analysing the changes in the composition of the anodic microbial population. The changes in abundance and diversity of the species in the microbial community in the electroactive biofilm and a comparison with non-MESs cultures allowed us to find a correlation between the composition of the community and the enhanced degradation capacity. Finally, we assessed the electrochemical performance of enriched anodic biofilms of MESs.

Materials and methods

Chemicals

All the chemicals including analytical grade glyphosate (Pestanal®) and potassium hexacyanoferrate (III) used in the present study were purchased from Sigma-Aldrich, UK. A stock solution of glyphosate (50 mg/L) was prepared dissolving the solid reagent in deionized water and stored at 4°C in dark conditions.

MESs configuration

The degradation of glyphosate was explored in two-chamber MES bioreactors operated in batch mode. The working volume of both cathode and anode compartments was 8 ml. Both anode and cathode electrodes (2.5 cm × 2.5 cm) were made up of carbon felt (Alfa Aesar, Haverhill, USA) and placed at equal distance from a cation exchange membrane (CMI-7000, Membranes Int., USA) separating the two chambers.

Replicate batch experiments (n = 4) were performed at room temperature (25 ± 5°C) to study the degradation of glyphosate in MESs. Anaerobic digestion sludge collected from Goddards Green Wastewater Treatment Works (Hassocks, UK), was used to inoculate the anode compartment. The culture medium contained (per litre of deionized water): Na_2HPO_4 (6.02 g), KH_2PO_4 (1.024 g), NH_4Cl (0.41 g), CH_3COONa (1.0 g), and mineral media (10 ml). The composition of mineral media (per litre of deionized water) was $CoCl_2{\cdot}6H_2O$ (0.082 g), $CaCl_2{\cdot}2H_2O$ (0.114 g), H_3BO_3 (0.01 g), $Na_2MoO_4{\cdot}2H_2O$ (0.02 g), $Na_2WO_4{\cdot}2H_2O$ (0.01 g), $MgCl_2$ (1.16 g), $MnCl_2{\cdot}4H_2O$ (0.59 g), $ZnCl_2$ (0.05 g), $CuSO_4{\cdot}5H_2O$ (0.01 g), and $AlK(SO_4)_2$ (0.01 g) [3]. Nitrogen gas was sparged into the culture medium for 3 min to attain an anaerobic environment. The catholyte solution contained (per litre of deionized water) 4 g potassium hexacyanoferrate (III) and 100 ml PBS buffer and was replaced at the end of each feeding cycle to avoid acidification of the medium and its negative effect on current generation.

Enrichment and acclimatization of the anodic microbial community

MES bioreactors containing 10 μg/l glyphosate were operated initially as a microbial fuel cell with an external resistance of 1000 Ω, with an initial concentration of 10 μg/L glyphosate. The concentration of glyphosate was gradually increased to 500 μg/L with a weekly feeding regime lasting two months. After the acclimatization process, the bioreactors were switched to microbial electrolysis cells to study the effect of applied voltage on glyphosate degradation. For this aim, an external resistance of 10 Ω was used while different voltages (0–500 mV) were applied to the bioreactors. The acclimatized community is referred to as MES-enriched culture.

Analytical methods

At the end of each feeding cycle (one week), the anolyte solution was collected for analysis and the anode compartment was replenished with fresh medium. The anolyte samples were filtered using 0.2 μm membrane filters (CHROMAFIL® Xtra, Macherey-Nagel, Germany) and stored at −20°C for further analysis. Glyphosate concentrations were measured using a glyphosate ELISA kit (Abraxis, Eurofin Technologies, Hungary). Glyphosate degradation was measured under different operational conditions: MESs with different applied voltages; microbial fuel cell at open circuit (where anode and cathode compartments are not connected); abiotic electrochemical reactor (i.e., a MESs without inoculation); and in a microbial culture where the initial inoculum was inoculated in a conventional bioreactor, to control for non-bioelectrochemical degradation.

Electrochemical analysis

The electrical output of the MESs was recorded every 2 min using a data acquisition system (Pico Technology, Cambridgeshire, UK) connected to a personal computer. Current generation was calculated by Ohm's law, I = V/R, where V is the reactor voltage and R is the external resistance (1000 or 10 Ω). Linear Sweep voltammetry (LSV) analysis was carried using a potentiostat with a scan rate of 5 mV/s, to study the activity of electroactive biofilms with and without applied voltages (PalmSens PS4, PalmSens, Houten, Netherlands). Prior to LSV analysis, the anode chamber was filled with fresh medium, the catholyte solution was replaced, and the MESs were kept under open circuit conditions for 2 h.

Microbial community analysis

Total genomic DNA was extracted from the anodic biofilms, initial inoculum, and non-MESs cultures, using a DNeasy® PowerSoil® Pro Kit (Qiagen, Germany). To extract DNA from anodic biofilms, the MESs were disassembled at the end of operation, and the whole anode was used. The quality of DNA samples was analysed by Nanodrop (ThermoFisher Scientific), and 16S rDNAamplicon sequencing was performed (Novogene (UK). For amplicon sequencing, genes of distinct regions (16SV4/16SV3/16SV3-V4/16SV4-V5) were amplified used specific primers (e.g. 16S V4: 515F-806R) with the barcode. Library generation was performed using NEBNext® UltraTM DNA Library Prep Kit for Illumina and quantified via Qubit and Q-PCR.

Phenotypic analysis

The enriched anodic community was studied using Biolog microplates. Biolog (MT2) 96-well microplates (Biolog, Hayward, USA) were used to assess the ability of initial inoculum and enriched anodic cultures to utilize glyphosate as a carbon source. Each well contains nutrient medium (except carbon source) and cellular respiration is measured spectrophotometrically by the reduction of the redox dye tetrazolium violet. In the present study, MT2 microplate assay was used

to confirm if the microbial enrichment in MESs resulted in the enrichment of glyphosate-degrading microbes, which can use glyphosate as a sole carbon source. Microbial samples were extracted either from anodic biofilms or initial inoculum (sludge), washed three times and resuspended in sterile water. 15 µl of a 2 % glyphosate stock solution (0.3 mg of glyphosate) was added to each well. Resuspended inoculum or enriched microbial cultures (150 µl) were then inoculated into each well (triplicate). Microplates were incubated anaerobically at room temperature. The absorbance of the wells at different wavelengths (540, 565, 590, and 600 nm) was determined every 15 min using a Clariostar microplate reader (BMG LABTECH). The average well colour development (AWCD) curve was plotted after normalizing the absorbance of each well against the reading of the water-containing well. The negative values were considered zero [23].

Results and discussion

Glyphosate degradation

The degradation of glyphosate by MESs and non-MESs enriched cultures was assessed using ELISA. In Fig. 1, the residual glyphosate measured in the reactors is presented, while Table 1 shows the degradation of glyphosate as % of the initial concentration in the reactors.

The results from the control show that glyphosate is not broken down spontaneously in solution, confirming its stability and long half-life [20], while the reduction of glyphosate caused by a natural microbial community is around 50 %. In soil, the main mechanism of of glyphosate degradation is the activity of soil microorganism [38], with reported half-lives ranging between 7 and 60 days [15]. Microbial activity is known to play an important role on the degradation of glyphosate in aqueous environments, although its persistence is also variable, as it depends on the presence of suspended solids, the movement of the water, and other environmental parameters [17,13].

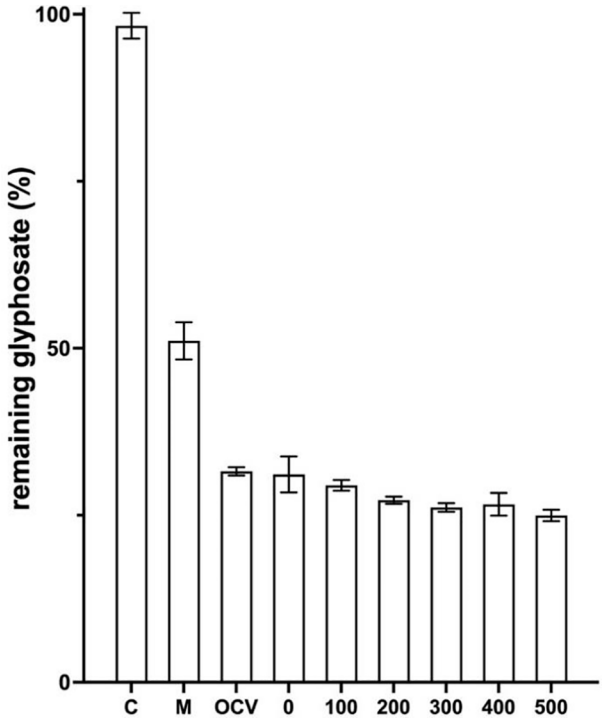

Fig. 1. Residual concentration of glyphosate in MES. C: Abiotic control; M: Microbial culture (where the initial inoculum was inoculated in a conventional bioreactor); OCV: Microbial fuel cell at open circuit (where anode and cathode compartments are not connected); 0 – 500: applied voltage (mV). Remaining glyphosate is the percentage of glyphosate remaining in the reactor after operation.

Table 1
Degradation of glyphosate as % of the initial concentration in the reactors.

Condition	% degradation
0 mV	68.89 ± 2.69
100 mV	70.51 ± 2.73
200 mV	72.75 ± 0.82
300 mV	73.84 ± 0.90
400 mV	73.36 ± 0.80
500 mV	75.03 ± 1.27
OCV	68.41 ± 1.22
Non-MESs (microbial cultures)	48.88 ± 0.51
Abiotic	1.76 ± 0.80

In MESs reactors, the degradation of glyphosate was significantly higher (ranging from 68.41 ± 1.21 % at 0 mV to 75.03 ± 0.79 % at 500 mV) than in non-MESs reactors (48.88 ± 0.51 %), and a slight increase in the degradation was observed with higher applied voltages. These results indicate that a system to treat wastewaters integrating microbial electrochemical systems could be essential to completely degrade residual glyphosate in waters.

The low degradation (3.12 %) compared to that observed under biotic conditions indicates that the contribution of abiotic degradation in MESs was insignificant compared to the degradation in the presence of an electroactive microbial community.

The presence of an electrode in MESs could affect the structure and composition of the microbial community different to that in a non-MESs, resulting in different performances between the two systems. Higher abundances of *Pseudomonas* (10.77 %) were observed in MES than in non-MES (2.11 %). Species of the genus *Pseudomonas* are known to degrade glyphosate.

In general, glyphosate degradation in MESs was slightly higher when a voltage was applied than the degradation without applied voltage, with differences ranging from 2.09 % to 5.49 %. The degradation at open circuit (OCV) was the same as the degradation at closed circuit without applied voltage (0 mV), confirming that the acclimatized community associated to the electrode is electroactive and can degrade glyphosate. Upon step increases of voltage, the degradation of glyphosate increased slightly but consistently with the applied voltage until reaching a value of 75.03 % at 500 mV. It is known that optimum voltages can be found for the microbial degradation of pollutants in MESs [2]. For example, an optimum voltage of 400 mV was determined for carbamazepine degradation in a MES, but higher voltages caused a decrease in carbamazepine degradation, a phenomenon attributed to the poor adaptability of anodic biofilm to high voltage [44]. In the case of glyphosate, the degradation showed a continuous increase with voltage, but further analysis is required to determine the optimum voltage.

In order to assess the contribution of the electroactive microorganisms to the degradation of glyphosate, is necessary to compare the degree of degradation of the MES with that of a process in a non-MES reactor. Only a few studies on the biodegradation of glyphosate using microbial communities from aquatic or terrestrial environments have been reported. As mentioned above, the half-life of glyphosate in most soils ranges between 7 and 60 days, depending on the microbial community and environmental conditions [15,22]. In a study, the ability of microbial communities extracted from different soils for glyphosate biodegradation was evaluated and reported a 50 % degradation of glyphosate (initial concentration of 50 µg/L) in 7 days [14]. In another study, half of the glyphosate was degraded in 9 days by downstream river microbial communities, dominated by *Phenylobacterium* sp. and *Starkeya* sp. [6].

There are two major metabolic pathways for glyphosate biodegradation in glyphosate-degrading microorganisms. In microorganisms that can use glyphosate as a source of phosphorous, glyphosate is converted into sarcosine by the activity of enzymes able to cleave

carbon–phosphorus (C—P) bonds. Sarcosine is subsequently degraded into carbon dioxide and water via different metabolic pathways. In microorganisms that use glyphosate as a nitrogen source, the metabolic pathway involves the conversion of glyphosate to amino-methylphosphonic acid (AMPA) and glyoxylate, by the activity of the enzyme glyphosate oxidoreductase, which splits the carboxymethylene–nitrogen (C—N) bond [19]. According to the literature, most of the *Pseudomonas* species isolated as glyphosate-degraders metabolize glyphosate via the sarcosine pathway [42].

The use of MESs for degradation of pesticides has been reported by a few studies, most of them using soil MFCs. The degradation of DDE (2,2-bis (p-chlorophenyl)-1,1-dichloroetylene), a persistent pesticide, in a soil MFCs operated for 2 months, 39.4 % of DDE was degraded in MFCs accompanied with a maximum power output of 30 μW [8]. In another study, soil MFCs were used for remediation of hexachlorobenzene, an organic pesticide, and 37.12 % of the compound was degraded in 21 days [9]. However, these cannot be compared to liquid MFCs.

Electrochemical characterisation of the anodic biofilm

The electrochemical performance of the anodic biofilm was characterised using LSV to further confirm the enrichment of electroactive microorganisms and study the correlation between glyphosate degradation and electrochemical properties. LSV is a simple and widely used technique to study the electron transfer by anodic electroactive bacteria and subsequently the electrochemical performance of MESs. The polarisation curve indicates the relation between cell voltage and current generation for the studied voltage range and enables the calculation of the maximum current potential for an electrochemical system. Results of LSV analysis for MESs operated as MFCs and MESs with applied voltages are shown in Fig. 2 and Fig. 3, respectively. For the MFCs (Fig. 2), the potential was swept linearly from open circuit voltage to 5 mV. The maximum current and power output obtained by MFCs were 217.03 mA/ m^2 and 92.81 W/m^2 respectively. An increase in the current was observed as the voltage swept from open circuit voltage to around 317 mV. No current increase was observed as the voltage swept beyond this point.

LSV was also performed for the MESs operated with different applied voltages (from 0 to + 500 mV), to study how anodic electroactive bacteria in each case respond to changes in cell voltage, and to determine the effect of the applied voltage on the electrochemical performance of MESs. To perform LSV analysis, the potential between the electrodes was swept linearly in time (starting from open-circuit voltage up to + 500 mV) and the current response was plotted as a function of potential (Fig. 3). An increase in the applied voltage from 0 to + 300 mV led to an increased maximum current generation from 422.03 to 611.95 μA. This

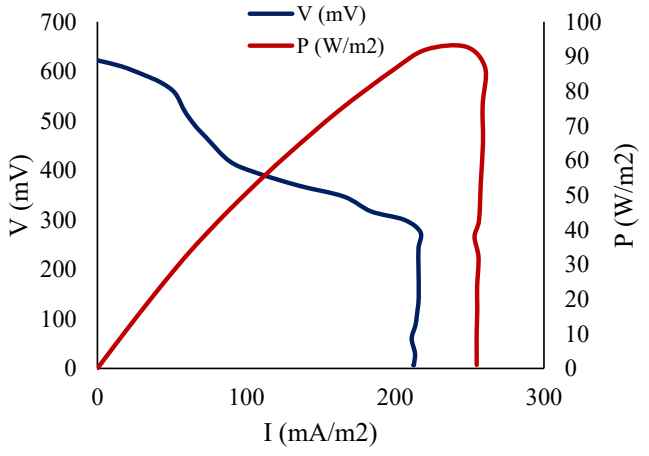

Fig. 2. Polarisation curves and power outputs for the MFCs containing 500 μg/L glyphosate. I: current; P: power; V: voltage.

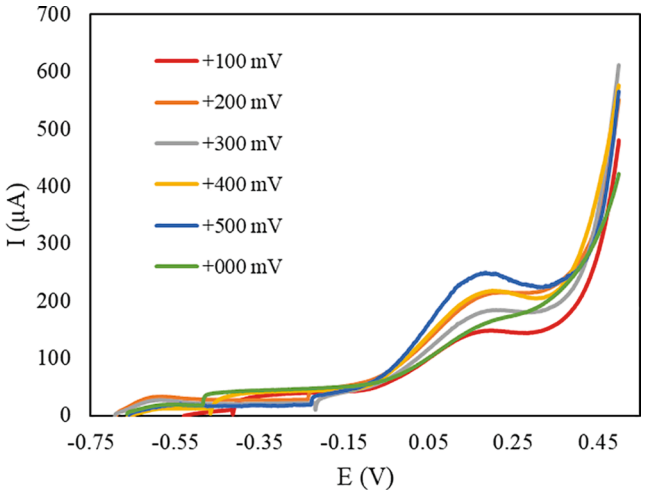

Fig. 3. Linear Sweep Voltammetry analysis for the MESs with different applied voltages.

current increase could be attributed to the enhanced degradation of glyphosate, which resulted in excess electrons. Further increase in the applied voltage beyond + 300 mV resulted in decreasing currents: the maximum currents for MESs operated with applied voltages of + 400 and + 500 mV were 575.68 and 565.44 μA, respectively. While the application of a potential stimulates EAB and therefore increase the current generation [25], voltages higher than a threshold value may reduce EAB activity due to changes in conformation, structure or redox properties of the enzymes involved, resulting in lower electrochemical performances [11].

Microbial community analysis

The microbial communities of the initial sludge, the anodic biofilms, and the non-MES control cultures were analysed by 16S rRNA high-throughput sequencing to: 1) determine the changes in the composition of the enriched microbial populations caused by exposition to glyphosate; 2) to study the variation between microbial species enriched in MESs and non-MESs reactors; and 3) to gain further insight on the possible pathways for degradation of glyphosate in MESs.

Table 2 shows statistical indices of alpha diversity when the clustering threshold is 97 %. The Goods coverage index for the initial sludge (0.993), MESs anodic biofilm (0.999), and non-MESs cultures (0.996) show that almost all the microbial populations and operational taxonomic units (OTUs) were covered by 16S rRNA sequencing.

The number of observed species, as well as the Chao1, Shannon, and Simpson indices indicate that the microbial diversity decreased after enrichment in MESs and in non-MESs reactors (microbial cultures) compared to the initial sludge (Table 2). Shannon diversity indexes of 3.531, 3.614, and 6.702, for the anodic microbial communities, the microbial cultures, and the initial inoculum, respectively, suggested that non-MESs enriched cultures were more diverse than MESs anodic biofilms. The lower diversities of enriched cultures could be attributed to the inhibition of glyphosate-sensitive species in both MESs and non-MESs reactors.

The results of microbial community analysis at phylum, class, and genus level are shown in Fig. 4. The initial community (inoculum) was dominated by five phyla, including *Euryarchaeota* (27.01 %), *Proteobacteria* (16.75 %), *Firmicutes* (14.86 %), *Cloacimonetes* (14.03 %), and *Bacteriodetes* (10.18 %). After the enrichment process, there was a large difference between microbial communities detected in anodic biofilms and non-MESs cultures. In both MESs and non-MESs enriched cultures, some phyla, including *Firmicutes*, *Cloacimonetes*, *Bacteriodetes*, *Chloroflexi*, and *Spirochaetes*, exhibited an obvious decrease compared to the

Table 2
Alpha diversity indices for different microbial samples.

Sample	Observed species	Shannon	Simpson	Chao1	ACE	Goods coverage	PD_whole_tree
Initial inoculum	2008	6.702	0.948	2278.905	2340.709	0.993	167.699
MES	428	3.531	0.722	458.373	475.508	0.999	39.000
Microbial culture	703	3.614	0.720	784.657	830.485	0.996	81.674

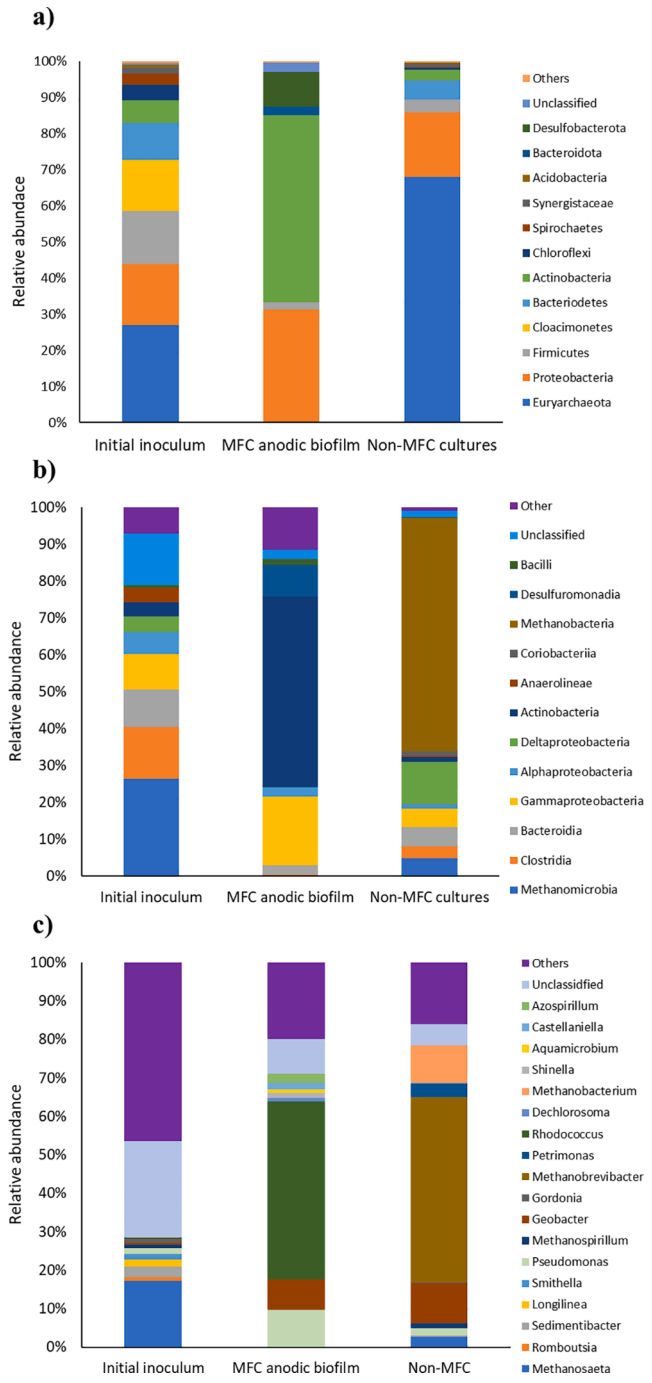

Fig. 4. Composition of microbial communities on MFC anodic biofilms and non-MFC reactors (microbial cultures) at a) phylum, b) class; c) genus level.

initial inoculum, attributable to their sensitivity to glyphosate. *Actinobacteria* (51.74 %) was the dominant phylum in anodic biofilms, while in non-MESs cultures, *Euryarchaeota* showed the highest relative abundance (68.06 %). The high abundance of archaea in non-MESs (in contrast with the observations in MESs) could be explained by the fact that in MESs, electroactive species compete with methanogens for hydrogen and outcompete them [1]. *Proteobacteria* was the second dominant phylum in both anodic biofilms (31.18 %) and non-MES cultures (17.73 %) (Fig. 4a). The selective enrichment of putative EAB in anodic biofilms was further confirmed at class level with increased relative abundances of *Actinobacteria* and *Gammaproteobacteria* (about 12.8 and 2-fold increases compared to the initial sludge, respectively) (Fig. 4b). *Actinobacteria* (51.68 %), *Gammaproteobacteria* (18.67 %), and *Desulfuromonadia* (8.67 %), were the three classes with highest relative abundances in anodic biofilms, while in non-MESs enriched cultures, *Methanobacteria* were predominant (63.2 %). Some classes including *Clostridia*, *Bacteroidia*, *Alphaproteobacteria*, and *Anaerolineae*, showed lower abundances in both MESs and non-MESs cultures, compared to the inoculum.

Genus level analysis (Fig. 4c) indicated that the anodic biofilm was dominated by three genera known for their electroactivity, including *Rhodococcus* (51.26 %), *Pseudomonas* (10.77 %), and *Geobacter* (8.67 %), while *Methanobrevibacter* (51.18 %), *Geobacter* (11.20 %), and *Methanobacterium* (10.32 %) had the highest relative abundances in non-MESs cultures. Interestingly, methanogens including *Methanosaeta*, *Methanobacterium*, and *Methanobrevibacter* were not detected in anodic biofilms, suggesting that EAB outcompeted methanogens. On the contrary, the three mentioned methanogenic genera were enriched significantly in non-MESs cultures, together accounting for more than 60 % of community. Members of the genera *Rhodococcus, Geobacter,* and *Pseudomonas,* are widespread in different environments including soil and marine sediments, and can consume a wide range of recalcitrant organic compounds as substrate [48]. In addition, members of these three genera have been detected frequently in MESs [4,41]. Several of the most common glyphosate-degrading species belong to the genus *Pseudomonas* [49]. It has been reported that the abundance of *Pseudomonas* is related with degradation of glyphosate in soils [16].

Unclassified genera accounting for 10.11 % and 5.90 % in anodic biofilms and non-MESs cultures respectively, may also have important roles in glyphosate degradation. Other identified genera that have currently been isolated as glyphosate-degraders include *Achromobacter*, *Agrobacterium*, *Comamonas*, *Achromobacter*, *Ochrobactrum*, *Geobacillus* and *Rhizobium* [30,49]. In the present study, none of these genera were found in the initial inoculum nor after microbial enrichment.

Our data shows that some of the electrogenic bacteria including *Rhodococcus, Geobacter,* and *Pseudomonas,* could tolerate glyphosate and be enriched in MESs. These genera have been previously reported to participate in the biodegradation of other pollutants including antibiotics, herbicides, pharmaceuticals, and aromatic compounds [21,47,51].

MT2 microplate assay for glyphosate utilization by anodic communities

MT2 assay is an inexpensive and effective method to evaluate the ability of inoculated microorganisms to utilize selected carbon sources. An MT2 microplate assay was carried out to further study the bioactivity of anodic biofilm for glyphosate utilization. The AWCD values of the glyphosate utilized by anodic microbial community was significantly higher than those of the initial inoculum (Fig. 5). This suggest that the microbial enrichment in MESs resulted in enhanced metabolic activities for glyphosate utilization. The complementary results obtained from MT2 microplate assay, together with glyphosate degradation values

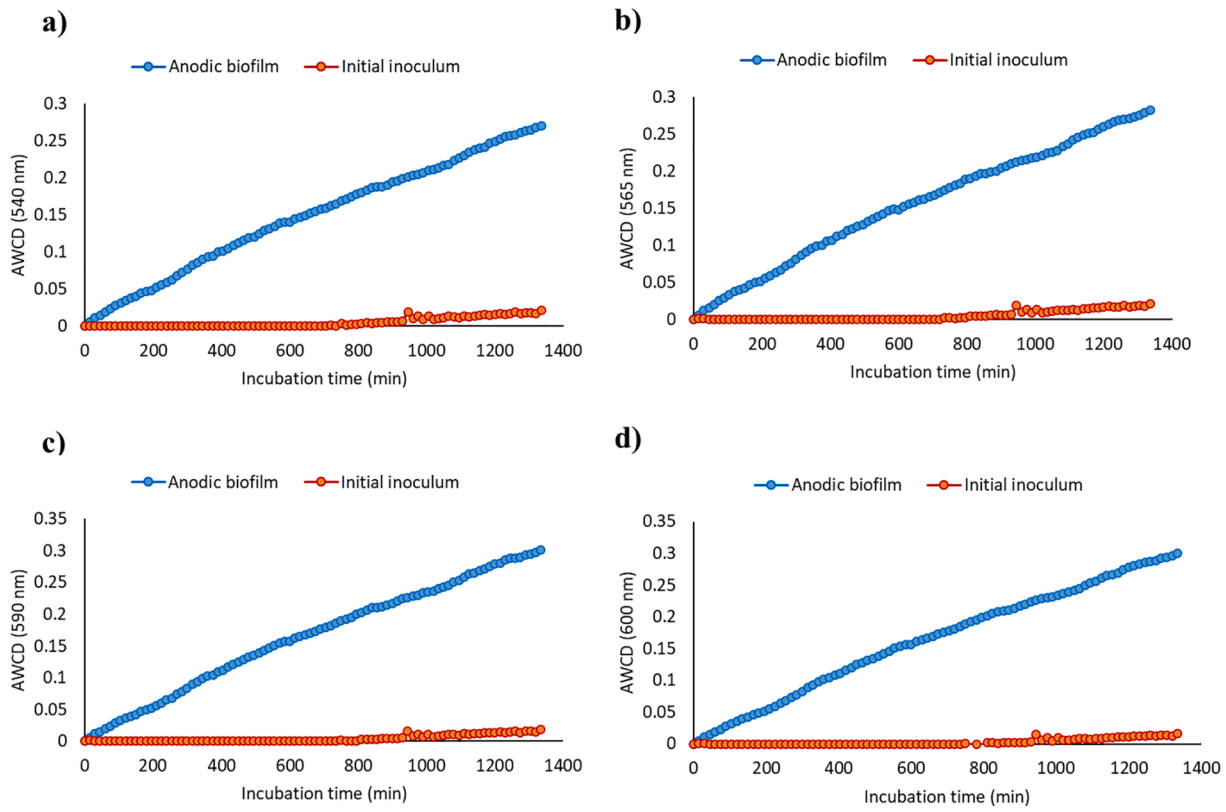

Fig. 5. Average well colour development (AWCD) at different wavelengths for glyphosate utilization by the initial inoculum and the enriched anodic biofilm a) 540 nm; b) 565 nm; c) 590 nm; d) 600 nm.

obtained by ELISA, confirmed that MESs operation resulted in enrichment of microbes that are involved in glyphosate degradation. Moreover, MT2 microplate assay revealed that anodic microbial communities could grow on glyphosate as a sole carbon source.

Conclusions

The present study reported for the first time the capacity of MESs to enhance the degradation of glyphosate. The preliminary results suggested than MESs could support higher degradation of glyphosate (up to 75 %) compared to microbial cultures (ca. 49 %). The significantly low degradation of glyphosate in abiotic conditions (3.12 %), revealed the enhanced degradation of glyphosate in MESs could be attributed to the difference in microbial community composition. Microbial community analysis revealed that abundant genera in anodic biofilms of MESs were *Rhodococcus*, *Pseudomonas*, and *Geobacter*, while the microbial community enriched in non-MESs reactors, was dominated by methanogens (*Methanobrevibacter* and *Methanobacterium*).

Our study shows the enhanced degradation of glyphosate using MESs. To further assess the fate of glyphosate and identify or confirm its biodegradation pathways by a microbial community, and the mechanisms involved, the expression levels of the genes related to different degradation pathways could be monitored. In addition, isotope labelling could be used to trace its biodegradation metabolites.

The combination of MES with other advanced oxidation processes could result in the complete elimination of glyphosate from polluted wastewaters.

CRediT authorship contribution statement

Razieh Rafieenia: Investigation, Data curation, Conceptualization, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Mohamed Mahmoud:** Conceptualization, Methodology, Writing – review & editing. **Fatma El-Gohary:** Conceptualization, Funding acquisition, Project administration, Writing – review & editing. **Claudio Avignone Rossa:** Conceptualization, Funding acquisition, Project administration, Formal analysis, Methodology, Writing – original draft Writing – review & editing.

Declaration of Competing Interest

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.

Data availability

Data will be made available on request.

Acknowledgments

RR, MM, FE-G and CAR were supported by a Newton-Mosharafa Fund Institutional Link Grant from the British Council (Grant no. 352368074) and the Science, Technology, and Innovation Funding Authority (STIFA), Egypt (Grant no. 30901).

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