Article

Bioelectrochemical Greywater Treatment for Non-Potable Reuse and Energy Recovery

Daniele Cecconet [1,2,*], Silvia Bolognesi [2,3], Luca Piacentini [2], Arianna Callegari [2] and Andrea G. Capodaglio [2]

1 Department of Chemistry, University of Pavia, via Torquato Taramelli 12, 27100 Pavia, Italy
2 Department of Civil Engineering and Architecture, University of Pavia, via Adolfo Ferrata 3, 27100 Pavia, Italy; silvia.bolognesi@unipv.it (S.B.); luca.piacentini02@universitadipavia.it (L.P.); arianna.callegari@unipv.it (A.C.); capo@unipv.it (A.G.C.)
3 LEQUiA, Institute of the Environment, Campus Montilivi, Universitat de Girona, C/Maria Aurèlia Capmany 69, E-17003 Girona, Spain
* Correspondence: daniele.cecconet@unipv.it

Abstract: Greywater normally represents the largest fraction of wastewater generated in buildings and may be suitable for non-potable reuse after on-site treatment. Conventional technologies for greywater treatment include sequencing batch reactors, membrane filtration, and membrane biological reactors. Even though these can be very effective, they are highly energy consuming and may negatively impact the energy balance of the building where they are installed. Microbial fuel cells (MFCs) have emerged as a sustainable technology for contaminant removal and energy production from a variety of substrates. In this study, the application of MFCs for greywater treatment is reported, with a particular focus on the analysis of energy losses, in view of non-potable reuse. MFCs were fed with different types of greywater, characterized by either high or low conductivity, because greywater's conductivity may greatly differ based on its origin; in either case, organic matter (chemical oxygen demand; COD) removal was higher than 85% and not influenced by the influent conductivity, coupled with a maximum power production of 0.46 mW L^{-1} and 0.38 mW L^{-1}. Electrolyte overpotentials were dramatically higher in the case of low conductivity greywater (20% vs. 10%, compared to high conductivity influent); these overpotentials are related to the conductivity of the influent, showing that low conductivity hindered energy generation, but not COD removal. Polarization and power curves showed higher internal resistance in the case of low conductivity, confirming the overpotentials' analysis. Results showed the feasibility of the use of MFCs in greywater treatment, with potential to reduce the energy demand connected to its reuse compared to conventional technologies; coupling with a disinfection stage would be necessary to fully comply with most non-potable reuse regulations.

Keywords: greywater; non-potable reuse; microbial fuel cell; bioelectrochemical systems; bioenergy; conductivity

1. Introduction

Water scarcity and resource recovery issues have led to the formulation of innovative water paradigms, with particular focus on decentralized on-site treatment [1], source separation [2], and fit-for-purpose reuse [3]. Furthermore, as a consequence of emerging concerns about water cycle energy use and greenhouse gas (GHG) emissions [4–6] proper technology selection and valorization of often-neglected waste streams are becoming crucial issues. Source-separated greywater (GW) could contribute to lower the demand of valuable drinking water currently utilized for non-potable uses, greatly reducing domestic per capita water consumption. It is estimated that a fraction between 60% and 75% of domestic wastewater is represented by GW [7–9], defined as used water from domestic sources, including kitchen sinks (but excluding waste grinders, if present), washing machines, and hand basins, with the exclusion of toilets, bidets, and urinal flows [10]. Its amount

could be quantified in up to 100–150 L/PE/day in high-income countries [9]. Compared to unsegregated domestic wastewater, GW contains a relatively lower amount of organic matter that needs to be removed in order to comply with applicable regulations regarding non-potable water reuse applications [11].

Current options for on-site GW treatment and reuse include membrane bioreactors (MBR) [3,12,13], sequencing batch reactors [14], and green walls [11]; nature-based solutions, such as constructed wetlands [15,16], are applicable, but they demand large spatial footprints. Innovative proposed technologies include electrocoagulation and filtration combined with biological processes [17]. All these options should consider potential uses and possible risks related to pathogenic and pollutants' residual content after treatment [18]. Among different options, membrane filtration and its coupling with biological treatment, MBR, are particularly common due to their simplicity, compactness, and solid-free effluents [3,13]. This makes them suitable for onsite application in single buildings or groups thereof, even in dense conurbations. However, some of the cited technologies are highly energy demanding: values of 0.16–0.24, 0.5–0.7, 0.1–0.3, and 0.11–0.22 kWh m^{-3} were measured for biological aerated filter, MBR, sequential batch reactor and conventional activated sludge, respectively, operated at pilot scale for urban non-potable reuse of greywater [19]. Matos et al. [20] reported energy consumption up to 1.89 kWh m^{-3} for MBR treatment of greywater in decentralized in-building applications. Therefore, this aspect may negatively impact the local energy and emissions balance of the installation site [21]. Therefore, alternative technologies that use less energy and may even generate it ought to be evaluated to enhance sustainability of GW treatment and its local reuse.

Bioelectrochemical systems (BESs) have emerged in the last two decades as a multifaceted platform for a variety of purposes, including organic matter removal, nutrient recovery, hazardous site remediation, and groundwater treatment [22–25]. The first and most common declination of BESs is represented by microbial fuel cells (MFCs), in which organic matter is oxidized in an anodic compartment; electrons collected at the anode travel through resistance to reach the cathode, where they are used in a reduction reaction. Electrochemically active microorganisms act as catalysts for reaction evolution at anode and cathode [26–28]. MFCs proved proficient in treating a large variety of wastewaters, including agro-industrial effluents, swine wastewater, and nitrate-contaminated groundwater [29–32]. The presence of organic matter at high concentrations in the anolyte, however, may lead to the development of an undesired competition between electrochemically active microorganisms and methanogens; this phenomenon is limited at low organic concentrations, while it can be highly detrimental at higher organic matter concentrations [33,34]. Due to the low organic content of GW, MFCs may therefore represent an ideal, virtually zero-energy input process technology for decentralized GW treatment for reuse, while at the same time producing a certain amount of energy from an often-neglected waste stream. Decentralized systems, in fact, can not only mitigate the effects of wastewater disposal on the environment and public health, but may increase the ultimate local reuse of water [35,36].

In this study, an application of a double-chamber MFC for GW treatment for possible reuse purposes, focusing on organic matter removal, energy production, and internal energy losses, is presented, and analyzed.

2. Materials and Methods

2.1. MFC Setup

Three identical H-type MFCs were built, each with two 130 mL glass half-cells, as shown in Figure 1. The cathode and anode were composed of granular graphite (model 00514, diameter 2.5–5 mm, EnViro-Cell, Oberursel, Germany) inserted in a perforated plastic cuvette to allow dispersion-less, full contact between the graphite and anolyte/catholyte. The volume of each graphite electrode was 10 mL, with a distance between electrodes of 5 cm. A cation exchange membrane (CEM, CMI-7000, Membranes International Inc., Ringwood, NJ, USA) separated the anodic and cathodic chambers. Addition of electrodes

decreased the internal net cell volume in each chamber (net anodic chamber, NAC, and net cathodic chamber, NCC) to 110 mL. Air was supplied to the cathode using an air pump to provide oxygen as a terminal electron acceptor (TEA) for the necessary cathodic reduction. Mixing was provided at the anodes by magnetic stirrers. Anodes of the MFCs were inoculated using the effluent of a long-term operating parent MFC with the addition of CH_3COONa (1.5 g L^{-1}) as an organic matter source. Once inoculated, the MFCs were fed with acetate-based solution for 30 batch cycles. After this initial phase, the MFCs were operated in batch mode using GW as the influent. Each of the MFCs was equipped with 3750 Ω external resistance (R_{ext}); this value was chosen based on polarization and power curves observation conducted in the preliminary acetate-fed phase. A phosphate buffer solution (PBS, 10 mM, pH = 7), with the following composition: 0.507 g L^{-1} NaH_2PO_4, 0.819 g L^{-1} Na_2HPO_4, was fed to the abiotic cathodes.
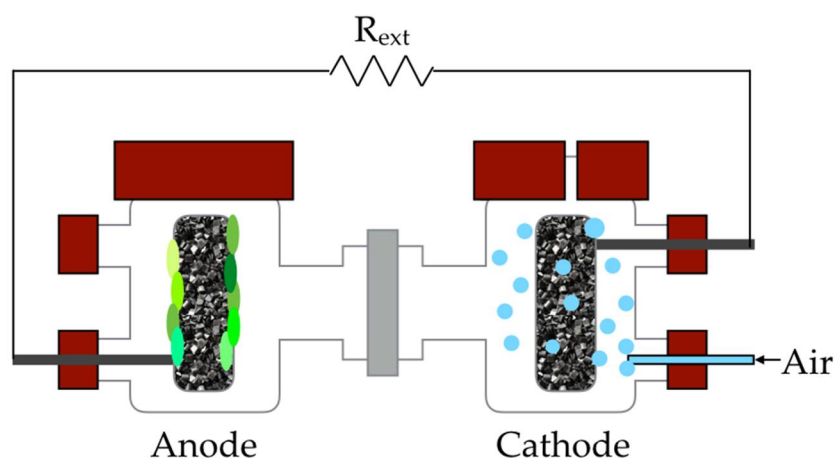

Figure 1. Scheme of the microbial fuel cell used in the experimentation. Air was supplied to the cathode using an air pump. R_{ext}: external resistance.

2.2. Greywater Composition

Due to different water consumption patterns, GW characteristics may greatly vary across geographical areas [37,38]. An analysis of reported physical and chemical parameters was conducted, leading to the definition of key GW characteristics summarized in Figure 2.

As shown in Figure 2, GW presents a wide range of conductivity values, which can greatly influence bioelectrochemical processes; variability in the ionic conductivity is attributed to the different initial uses of water [3]. Dissolved ions contribute to the charge transport and reduce the solution resistance [39,40]. Therefore, low ionic conductivity values may hinder electron and proton transport due to the increase in resistance in the BES reactor [41]. To proceed with the study, GW was prepared, modifying the recipe proposed by Jefferson et al. [42], consisting of handwashing soap (0.064 g L^{-1}), shampoo (0.8 mL L^{-1}), sunflower oil (0.01 mL L^{-1}), and effluent of a long-term operating MFC (2.4 mL L^{-1}). Details on shampoo and soap composition are available in the Supplementary Materials. The simulated GW was thus representative of average GW characteristics reported in the literature. A solution with higher conductivity was also prepared by KCl addition, and an acetate solution with a similar organic matter concentration with the following recipe: Na_2HPO_4 0.8192 g L^{-1}, NaH_2PO_4 0.5074 g L^{-1}, KCl 0.0026 g L^{-1}, CH_3COONa 1 g L^{-1}, NH_4Cl 0.02 g L^{-1}, micronutrients 1 mL L^{-1} was prepared as a control substrate. All the used solutions' parameters are summarized in Table 1.
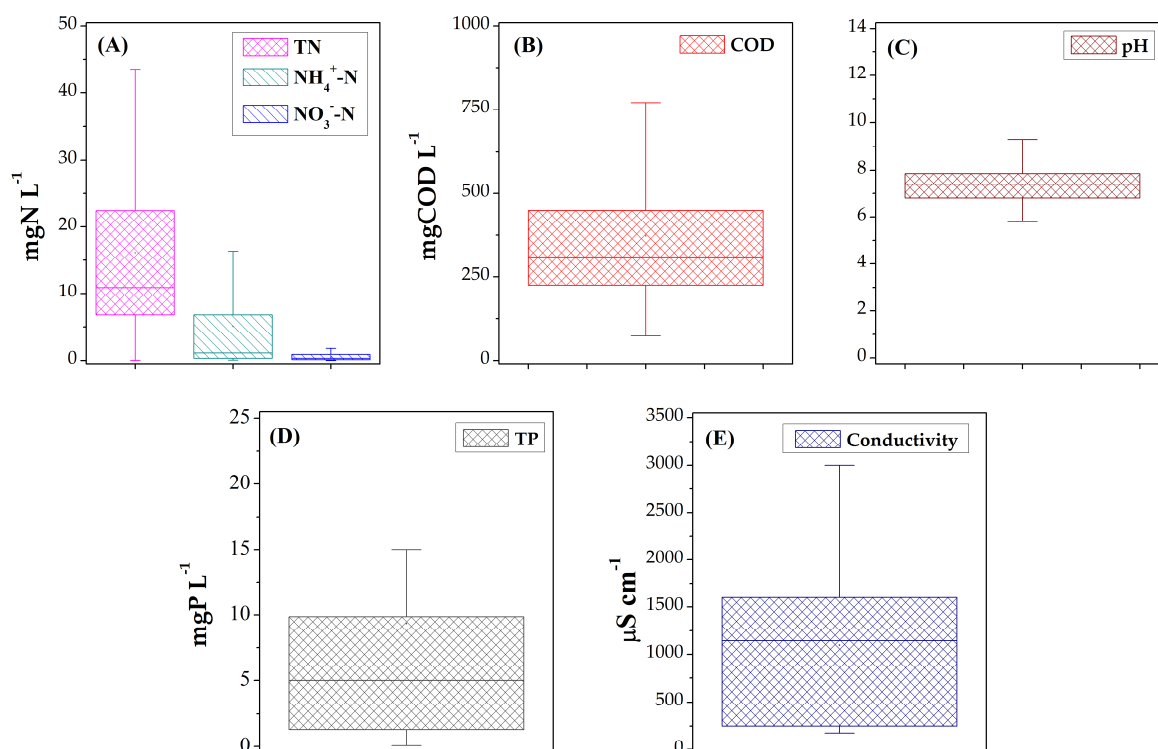
Figure 2. Chemical and physical parameters in greywater reported in the literature. Error bars represent the 25th and 75th percentiles. (**A**) Total nitrogen (TN), ammonia and nitrate nitrogen concentration; (**B**) Chemical oxygen demand (COD); (**C**) pH; (**D**) total phosphorus (TP); (**E**) conductivity. The list of the works consulted for the preparation of the figure is available in the Supplementary Materials.

Table 1. Characteristics of the synthetic microbial fuel cell (MFC) greywater (GW) influents. COD: chemical oxygen demand; TN: total nitrogen; TP: total phosphorus.

	COD mg L^{-1}	NO$_2^-$-N mg L^{-1}	NO$_3^-$-N mg L^{-1}	NH$_4^+$-N mg L^{-1}	TN mg L^{-1}	TP mg L^{-1}	pH -	Conductivity µS cm^{-1}
Greywater, low conductivity	489	0.1	0	0	1.8	1.13	8.65	348
Greywater, high conductivity	489	0.1	0	0	1.8	1.13	8.65	1578
Acetate solution	500	0	0	5.8	5.8	309.64	8.21	2360

After inoculation and start-up, three different performance tests were carried out, each in a different MFC fed with: (**a**) GW with low conductivity; (**b**) GW with high conductivity; and (**c**) acetate control solution. Each experiment lasted 500 h and was repeated three times to ensure reproducibility.

2.3. Monitoring and Analytics

Voltage, power, and electric current were monitored at 60 s intervals with an automatic data acquisition system (NI-USB 6008, National Instruments Co., Austin, TX, USA) connected to a computer. Influent characterization in terms of chemical oxygen demand (COD), NO$_3^-$-N, NH$_4^+$-N, total nitrogen (TN), and total phosphorus (TP) was carried out with a spectrophotometer (HI 83224 Wastewater Treatment Photometer, Hanna Instruments, Padua, Italy), while NO$_2^-$-N measurements were performed using nitrite test kits (HI3873, Hanna Instruments). All samples were stored at 4 °C prior to analysis for no

more than 24 h. Conductivity and pH were monitored at the beginning of each test using a multi-parametric probe (IntelliCAL^{TM} equipped with HQd^{TM} Digital Meter, Hach Lange, Lainate, Italy). At the end of each test (elapsed time 500 h), residual COD was measured. Current and power densities (mA L^{-1} and mW L^{-1}) were calculated by the ratio of the current or power and NAC. Normalized energy recovery (NER$_S$, kWh kgCOD$_{removed}$$^{-1}$) was calculated using Equation (1), as proposed by Ge et al. [43]:

$$NER_s = (P \cdot t)/\Delta COD \tag{1}$$

where P is the power (mW) measured by the data acquisition system, t is the time (s), and ΔCOD is the mass of COD removed in the batch (kgCOD$_{removed}$).

Polarization and power curves were obtained using a single-channel potentiostat (NEV4, Nanoelectra, Madrid, Spain) and imposing a linear decrease of 0.5 mV s^{-1} from the open circuit voltage (OCV) down to 0 mV. From these curves, internal resistance was calculated by the power density peak method [44]; electrochemical tests were conducted in a second phase (subsequent to the batch tests) in order not to affect the energy production during the tests [45]. The energy balance equation was used to calculate the energy loss factors, following the procedure reported in Sleutels et al. [46]. Anode and cathode overpotentials (η_{An} and η_{Cat}), ionic (E$_{ion}$), pH gradient (E$_{\Delta pH}$), and membrane transport losses (E$_t$) were then evaluated; ohmic losses other than ionic were not directly measured but included in the terms η_{An} and η_{Cat} [46].

3. Results

Each experiment was repeated three times; the graphs observed during the entire study are reported in Figure 3 and show good reproducibility of the results. When fed with low conductivity GW, MFCs achieved maximum voltage generation after a long (nearly 400 h) lag time in each batch, as shown in Figure 3A; this trend was confirmed in all tests with low conductivity GW. Maximum voltage of 346 mV was measured; at the end of each test, the voltage declined to about 100 mV. Current and power density trends during the experimentation are available in the Supplementary Materials (Figures S1 and S2, respectively).

Compared to the tests with high conductivity GW (Figure 3B), in the case of low conductivity GW, a much slower voltage increase, as well as lower observed peak voltage, are noticeable. This finding agrees with previously reported results, where electrochemical performances of MFCs were observed to increase with the ionic strength of the substrate [39,41,47]. Low wastewater conductivity usually causes overpotentials to increase, due to slow proton transport in the active biofilm [48] and higher resistance originating from the diffusion boundary and electric double layers [49,50]. In addition, low values of ionic conductivity were reported as a limiting aspect in the transfer of cations from anode to cathode and vice versa, therefore retarding the balance of electro-neutrality of the system [39,51]. Combination of these factors may lead to the observed decrease in MFC's energy production with low conductivity anolytes.

On the other hand, observed COD removal was not impacted by ionic strength differences, showing comparable values in both GW tests, and close to the values for acetate control test. Voltage in the control test reached a maximum value of 474 mV, higher than those observed when GW was fed as the anolyte. This could be attributed to GW complexity, due to the variety of compounds originally contained in detergents and personal care products, compared to the relatively simple acetate substrate, easily oxidable in the anodic compartment [52,53]. Complete removal of nitrogen and phosphorus from GW was observed at the end of each test.
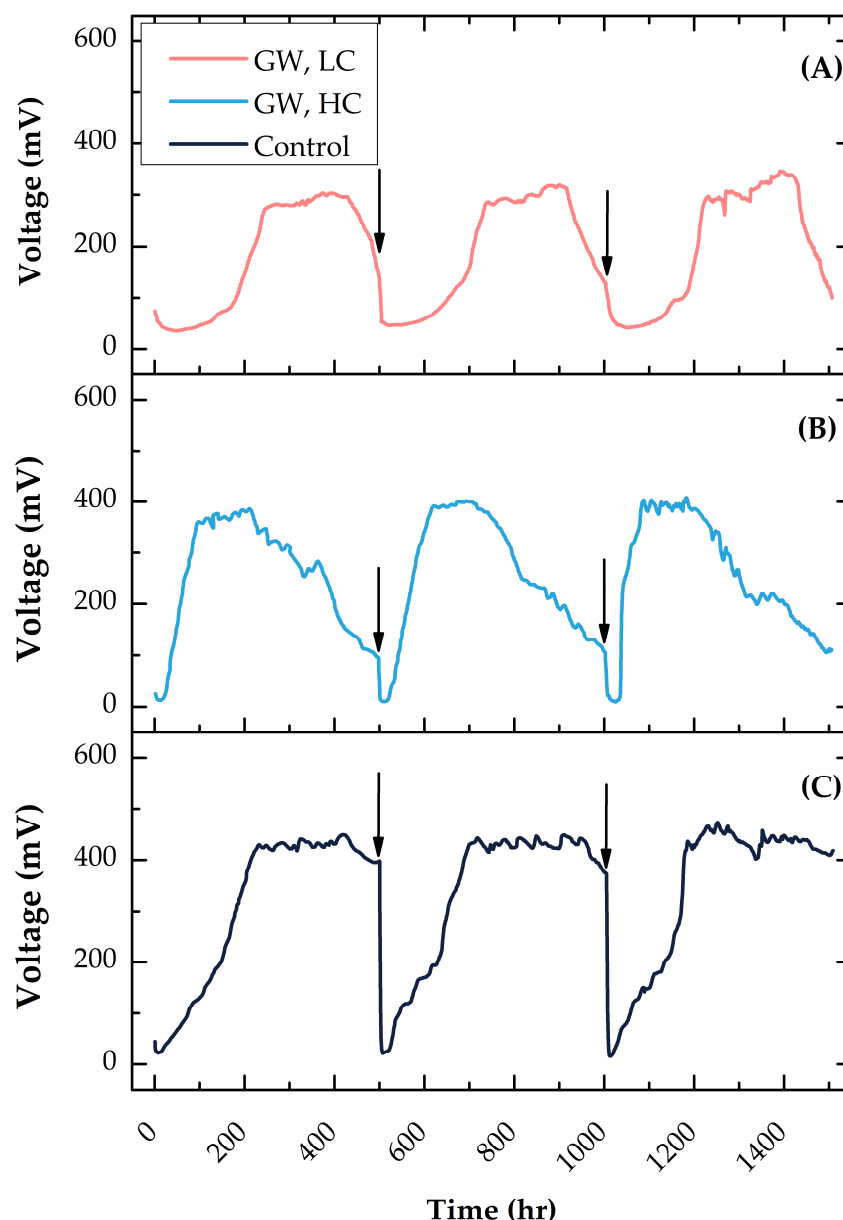
Figure 3. Voltage trend in the different tests. (**A**) GW, LC: greywater, low conductivity; (**B**) GW, HC: greywater, high conductivity; (**C**) Control: control test with acetate. Vertical black arrows represent the refresh of the MFC's medium at the end of a batch prior to the following.

Current and power densities reflected the trend set by voltage patterns, as displayed by the polarization and power curves shown in Figure 4. The MFC operated with high conductivity GW scored a P_{max} value 21% higher than that at low conductivity. Analysis of polarization curves allowed the calculation of the MFCs' internal resistance: 3323 Ω and 3740 Ω for low and high conductivity substrates, respectively; 2543 Ω for the acetate-fed control system. The use of GW as the influent led to the increase in internal resistances.

The analysis of the energy losses may suggest useful insights on the different performances of the MFCs fed with GW (low or high conductivity) or acetate. Several factors contribute the various losses and were considered in the analysis: anode and cathode overpotentials (η_{An} and η_{Cat}), conductivity-based ionic overpotentials (E_{ion}), pH gradient ($E_{\Delta pH}$), and membrane transport losses (E_t). As shown in Figure 5B, cathode overpotentials were identified as the major contributors in the overall losses, accounting for 42.2% in the case of the MFC operated with high conductivity GW, 37.6% in the case of low conductivity, and 45.0% in the control acetate-fed MFC. This may be attributed to the low performance

electrodes used in the cathode, in which abiotic conditions were maintained and no catalysts (Pt, Ti) were applied, paving the way for cathode overpotentials higher than 0.17 V, as shown in Figure 5A; the development of a biocathode would have led to a reduction in cathodic overpotentials [53,54]. In all the cases, the anode overpotentials reached values close to 30%, while pH-related overpotentials settled around 12%. Electrolyte overpotentials were dramatically higher in the case of low conductivity GW (20% vs. 10% in the other cases); these overpotentials are related to the conductivity of the influent [46], explaining why worse results in terms of energy production were scored when low conductivity GW was used as feed.
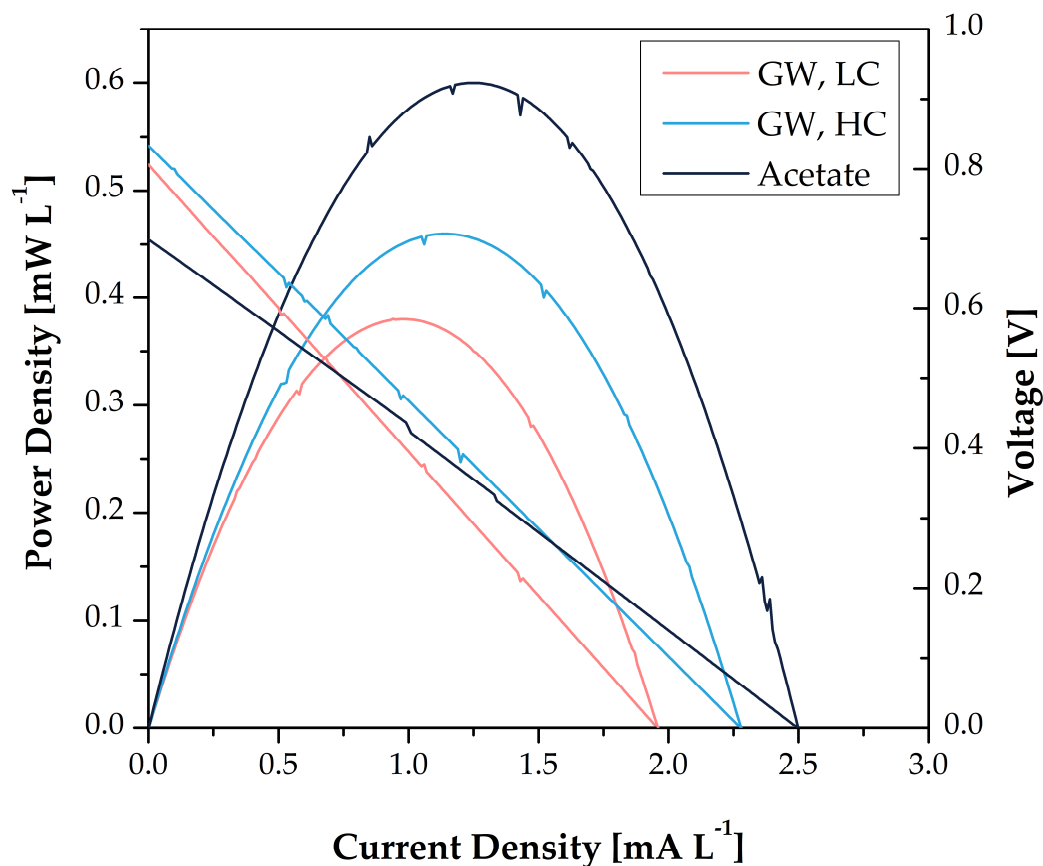

Figure 4. Polarization and power curves. GW, LC: greywater, low conductivity; GW, HC: greywater, high conductivity; acetate was used as anode feed in the control reactor.

Analysis of NER_s, a parameter expressing the amount of energy recovered from organic matter removal throughout the test normalized on the NAC, showed that high conductivity GW led to better values compared to low conductivity feed; results were lower than those reported in the control test with acetate, as shown in Table 2. Reported common NER values are in the order of < 1.0 in MFCs fed with different influents [43,55].
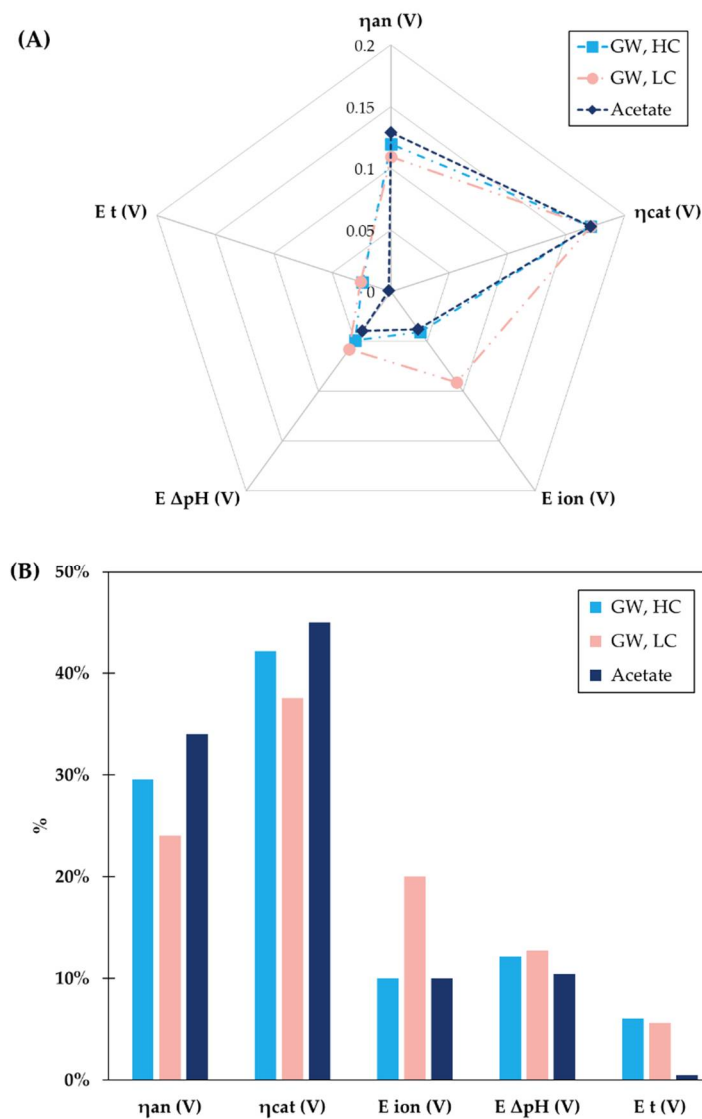
Figure 5. Energy losses in the different conditions. (**A**) distribution of the internal losses; (**B**) percentage of the internal losses. GW, LC: greywater, low conductivity; GW, HC: greywater, high conductivity; acetate was used as anode feed in the control reactor. E_t: membrane transport losses; $E_{\Delta pH}$: pH overpotential; η_{an}: anode overpotential; η_{cat}: cathode overpotential; E_{ion}: ionic overpotential.

Table 2. Maximum voltage measured, current density and power densities obtained from polarization curves, normalized energy recovery, and COD removal in the experimentation.

Test	NER_s kWh kg_{CODrem}^{-1}	CD_{max} mA L^{-1}	PD_{max} mW L^{-1}	η_{COD} %	V_{max} mV
Greywater, low conductivity	0.15	0.97	0.38	87%	346
Greywater, high conductivity	0.21	1.13	0.46	85%	409
Control, acetate	0.34	1.27	0.60	90%	474

4. Perspectives

As shown in Section 3, MFC technology can remove a large fraction (>85%) of the organic matter in GW. However, this might not be sufficient to ensure compliance of current

regulations for reuse, which usually require strict limits on coliform counts present in the treated effluents, even for non-potable-reuses such as toilet flushing [18,56]; this issue is shared with most conventional greywater treatment technologies [57]. MFC treatment should therefore be complemented by a disinfection stage to ensure sufficient pathogen reduction in effluents, as shown in Figure 6, although preliminary evidence suggests some disinfection capability by MFCs [58], an issue which deserves further experimental investigation. In addition, several greywater reuse regulations, including the U.S.A. [59], Canadian [60], Chinese [61,62], Japanese [63], and South Korean [64] regulations, require the presence of a chlorine residual in treated greywater intended for non-potable water reuse. Addition of chlorine-based disinfectants would then remove the presence of residual pathogens and ensure the necessary chlorine residual. Other regulations, such as the German [65], Italian [66], and Israeli [67], and World Health Organization guidelines [68], do not require the presence of chlorine residuals in the treated effluent to allow non-potable reuse. In these cases, an effective disinfection treatment could consist of UV disinfection: a stack of MFCs could partly sustain the energy requirement of a UV lamp system [69], allowing sustainable GW treatment. The development of LED UV lamps, with greater energy efficiency compared to traditional UV lamps, could improve the efficiency of similar setups. MFC/UV effluents water could then be safely used for many reuse applications. Batlle-Vilanova et al. [70] reported the simultaneous production of anodic methane and cathodic chlorine, which could be used as a disinfection agent; in the future, this may lead to a comprehensive GW treatment based solely on BESs.
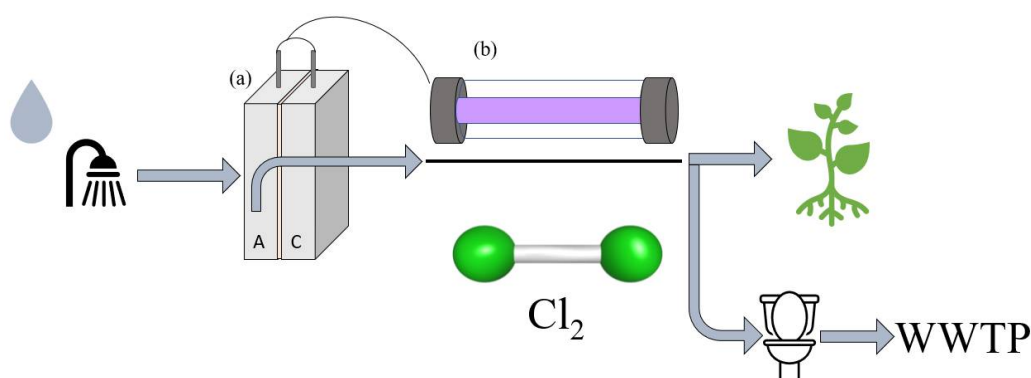

Figure 6. Schematic of the proposed on-site treatment plant. (**a**) MFC reactor; (**b**) disinfection stage, with UV lamp or chlorination. The effluent can be used for toilet flushing or for urban food crop cultivation. WWTP: wastewater treatment plant.

Bioelectrochemical reactions associated with power production (and related redox potential) were reported as the driving force behind the inactivation of different pathogens (*Salmonella enterica*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*) present in urine in a cascade system of MFC anodes connected in series [71,72]; a similar system succeeded in the removal of hepatitis B virus surface HBsAg and core HBcAg antigens, showing to reduce the possibility of environmental transmission of hepatitis B [73]. Connection in series of multiple BES units, proposed as an alternative to dimensional upscaling [74,75], may allow complete removal of pathogens, organic matter and nutrients, ensuring the safe reuse of greywater. Further research is necessary on this issue. Besides organic matter, GW contains a variety of other contaminants: fragrances, surfactants, emulsifiers, preservatives and antioxidants, softeners and plasticizers, UV filters, and solvents, other than estrogens and personal care products [7,76]. BESs have a proven capability to remove a wide variety of pharmaceutically active compounds and antibiotics [77,78], but their effectiveness on these pollutants has still to be proved in GW treatment schemes. Cross-circuit MFCs ought to be investigated to exploit both the anodic and cathodic redox environment. It was observed that surfactant addition led to an increase in MFC energy production in the treatment of bilge water, with no detrimental effects on electrochemically active microorganisms'

activity [79]; a similar result was obtained for the removal of PCB in contaminated soil using sediment MFCs [80]: in both cases, the increase in energy production was attributed to increases in the solubilization and bioavailability of the target compounds due to the surfactants' effect. The addition of the non-ionic surfactant Tween 80 to BESs showed contrasting results: addition to MFC's anode led to power production improvement due to the increase in cell membranes permeability, which in turn reduced electron transfer resistance [81]. No impact was shown on the cathodic activity of microbial electrolysis cells (MEC) at concentrations in the 5–20 mg L^{-1} range; decrease in current density was observed at a concentration of 80 mg L^{-1} [82]. The literature suggests that surfactants can be successfully removed using BESs, and that their addition may stimulate the removal of inorganic and organic compounds; however, they may exert toxicity effects negatively affecting biological and electrochemical performances [83,84]. Observations on conventional biological processes showed detrimental effects of anionic surfactants (2 mg L^{-1}) on organic matter and nitrogen removal, possibly due to their interference with oxygen transfer [85]. Reports of negative effects on MFCs' anodic microbiota at increasing sodium dodecyl sulfate (SDS, widely used in soaps) concentrations from 10 to 50 mg L^{-1}, leading to decrease in power production have also been published [86]; however, Chakraborty et al. reported 76.1% SDS removal in the anode of an MFC spiked with 10.21 mg SDS L^{-1}, simultaneously with 88% COD removal and steady power production [87]. Therefore, surfactant removal should be further explored, to ensure efficient process performance and foam-less, reusable treated water.

5. Conclusions

In this study, maximum power density of 0.46 mW L^{-1}, coupled with 85% COD removal were achieved through GW treatment by MFCs. Low GW conductivity, similar to that observed with other anolytes, limited energy production due to increased cell overpotentials (20% vs. 10% when compared with high conductivity) as revealed by the in-depth analysis of the energy losses; COD removal (and hence reuse possibilities) was not affected by the electrolytes' conductivity.

Results from this study suggest the feasibility of achieving satisfactory GW treatment with MFCs as a step leading to subsequent reuse, given the observed quality parameters of the effluent, in line with those expected from conventional wastewater treatment. Source-separated GW could then become an on-site, readily reusable resource, without the need for energy-intensive long-range transport to distant wastewater treatment facilities. As in any other resource reuse situation, the type of reuse would be subject to local regulations and other quality limitations, which would equally affect any type of used water treatment train. Low GW conductivity may limit bioelectrochemical energy production; however, considering that between 60% and 75% of domestic wastewater is represented by GW, segregated stream systems could contribute to safe sustainable local reuse, avoiding wastewater over-dilution that impacts negatively on water cycle energy demand, and on the generalized adoption of sustainable water management paradigms.

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