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Optimization of Microbial Fuel Cell Operation using Danube River Sediment

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Abstract

One of the main global focuses of mankind today is the required switch to new energy sources. Generating energy from waste is one of the potential solutions that can be achieved using microbial fuel cells (MFCs). Microorganisms, thanks to their ability to degrade organic substrates in contaminated environments, could contribute to solving our pollution challenge. The aim of this study was to investigate the potential of sediment with its natural microbiota from the River Danube to optimize electricity generation using MFCs. 16S rRNA gene analysis identified the main bacterial genera in the river sediment, *Clostridium*, *Bacillus* and *Tepidibacter*, which were isolated and cultured in the laboratory. Addition of these cultured microorganisms to the MFC resulted in current density of 192 mA/m^3, while the power density was about 8.80 mW/m^3. Our study confirms proper selection and enrichment of the microbial community can optimize the amount of current obtainable from river sediment by MFCs.

Keywords: microbial fuel cells, new energy sources, sediment from Danube River, free energy, power generation

1. Introduction

Global warming has been a main problem for years, but lately, the world has also been facing a lack of energy (Kumar et al., 2019). At the United Nations Climate Change annual meeting (UN Climate Change, 2019), the UN tried to draw attention to energy challenges, and they emphasized that the main area of interest, in recent years, is renewable energy sources (Kebir et al., 2019). Renewables cannot replace conventional fossil fuel-based energy sources, but using them in the right way, they can become remarkable sources of alternative energy (Slate et al., 2019).

Among several well-known options for green energy, microbial fuel cells (MFCs) are attracting attention in the last few years. It should be noted that MFCs are still considered a new technology despite the fact that the concept has been known for more than a century (Logan, 2009.)

One of the main advantages of MFCs is their ability to simultaneously contribute to solving two problems, i.e., organic waste degradation and our need for electrical energy production by exploiting organic waste (Tommasi and Lombardelli, 2017). MFCs, thanks to their principle of operation based on generating electricity from waste, are environmentally eco-friendly, sustainable and likely to be applied more extensively in the future in many fields (Li et al., 2019). The amount of energy produced by a MFC depends on several factors: the quantity of organic matter present in the waste material treated, the share of microorganisms that are capable of biodegrading the waste and effective electron transfer between the anode and the cathode (Xin et al., 2019).

There are several types of MFCs, depending on the complexity of their construction (one-chamber, two-chamber or multi-chamber systems), but all of them work in the same way (Najafpour, 2015). In general, contaminated sediment or some other source of organic materials is placed between the two electrodes. In the anodic compartment, electrochemically anaerobic bacteria use organic material and release electrons which are transferred to the electrode. In a previous study, Guo et al. (2019) showed that electrons flow through into a cathode compartment. At the same time, protons travel to the cathode compartment carried by fluid, where, together with electrons and oxygen, they build water. The potential difference between the electrodes is responsible for current formation (Corbella et al., 2015; Doherty et al., 2015).

Different types of microorganisms can be used in MFCs. Initially, microorganisms such as *Proteus mirabilis, Lactobacillus plantarum and Escherichia coli* were used. In MFCs, these bacteria released electrons, but for electron transfer to the anode, the presence of a mediator was required (Schröder, 2007). However, many mediators harm bacterial cells. Discovery of microorganisms that can transfer electrons to the anode without the mediator turned out to be necessary. Because of their characteristics, Logan (2009) defined these microorganisms as exoelectrogens, while other researchers also describe them as anode respiratory bacteria and electrochemically active bacteria. *Rhodoferax ferrireducens, Shewanella putrefaciens IR-1* and *Geobacter metallireducens* are examples of such microorganisms. Often, inoculated mixed cultures of microorganisms are used to diversify bacterial utilization of different organic compounds in the sediment, thus releasing more electrons and generating more current (Najafpour, 2015; Parkash, 2016).

Aquatic ecosystems have major environmental impacts. It is well known that concentrations of persistent and toxic substances are up to several times higher in river sediments than in waters, regardless of whether the main pollution source is in the water itself (Milenković et al., 2005; Rusina et al., 2019). However, river sediment is also a very important source of valuable organic matter and nutrients (nitrogen and phosphorus) - valuable from the perspective of fertilizing properties as well as the production of energy. The Danube, the second-longest river in Europe and one of the most important transport routes (Kirschner et al., 2009; Beškoski et al., 2013), flows through Serbia with a length of 588 km. (Lenhardt et al., 2016). Unfortunately, a significant amount of organic pollutants are carried into Serbia by the river and its sediment, but on the other hand, many of them originate from the local population (Kašanin-Grubin et al., 2019; Beškoski et al., 2013). Contaminants present in river sediment are most commonly of organic origin, containing N and P (Wang et al., 2019a). A build-up of these kinds of pollutants over long times leads to low levels of dissolved oxygen in the sediment, which creates an unpleasant odour that originates from the activity of sulphate-reducing bacteria (SRB) which could be potentially used for the electricity generation in MFC (Wang and Jiang, 2019b).

The aim of this study was to explore the use of MFCs for electric energy production from Danube river sediment. The voltages in MFCs were measured over time, followed by electrochemical calculations to obtain the current and the generated power. The microorganisms in the anode were first isolated and characterized, then utilized in MFCs to determine the effect of the additional bacteria on power generation.

2. Materials and methods

2. 1. Sampling and chemical properties of river sediment

Sediments used to construct MFCs were collected from the Danube in July 2018. The level of the river water was low and a malodorous smell was present. Sampling spots were positioned at the following location: 44°49’22.0”N 20°26’32.7”E, at the confluence of the Sava River into the Danube. A total of 20 sediment samples were collected and transferred into the laboratory. A composite sediment was made from all collected samples and was used in further work. Until use, composite sediment was stored in the refrigerator at 4 ^{o}C.

The original composite sediment was analyzed for pH and contents of moisture and total organic substances (Wilke, 2005). The total contents of C, H, N and S were determined on an automatic analyser (Vario EL III, CHNS/O, Elemental, Hanau, Germany). The carbonate content was analysed by the Rump method described earlier (Rump, 2000).

The content of metals in the sediment was determined by Inductive Plasma Mass Spectrometry (ICP-MS) (iCAP Qc/SN02767R Thermo Scientific, United Kingdom 2014) with a measuring range > 0.0003 μg/L and using software version 2.6.2270.44 (32 bit). Each determination was performed three times and the average value was used.

2.2. MFC experimental setup

Details on the design and construction of the single-chamber MFCs were published previously (Randjelović et al., 2019). Rectangular anodes and cathodes with a surface area of 80.3 cm^2 (11 cm x 7.3 cm) were formed for each MFC from inox mesh provided by Fasil, Serbia. Inox complying with IC 316 standard was chosen in order to maintain an inert ambient in the MFCs because of the direct contact the electrodes had with the sediment. Electrical contacts for the electrodes were formed from inox foil (Goodfellow, England) in the shape of rectangular extensions partly covered by heat-shrink hose, in order to assure electrical isolation. Together with the sediment, which was placed between the electrodes, each MFC was positioned separately in a plastic container with dimensions 16.8 cm x 10.8 cm x 6.0 cm. The distance between the two electrodes was filled with river sediment and was 2 cm, and the total volume of each MFC was 363 cm^3. The same composite river sediment was used in all MFCs. Four MFCs cells were formed:

- MFC_0 – control;
- MFC_I – unadjusted composite sediment;

- MFC_{II} – biostimulated composite sediment;
- MFC_{III} – biostimulated and bioaugmented composite sediment.

MFC_0 contained sterile composite sediment (composite sediment autoclaved for 25 min at 121°C) between the electrodes. Given that the power produced by a MFC is due to the biochemical processes of microorganisms, it was necessary to characterize the sterile MFC_0 immediately after construction. MFC_0 served as an abiotic control for other completed MFCs. MFC_{II} was prepared from composite sediment with added 1% (*w/w* in total sediment volume) sulphates (Na_2SO_4) and carbonates (Na_2CO_3), to stimulate indigenous microorganisms, and which had been stored for one month in an anaerobic atmosphere at 28 °C. MFC_{III} contained sediment with the same additives and stored under the same conditions as for MFC_{II}, but was also bioaugmented with three chosen SRB that had been previously isolated from the composite sediment and grown in laboratory conditions. $MFC_{I,}$ MFC_{II} and MFC_{III} were characterized over time, from five to twelve days after setup. Voltage was measured once daily at the ambient temperature of 25 °C. Loss of water by evaporation was controlled daily by weighing each MFC and adding sterilized demineralized water to maintain the initial weight of each MFC.

2.2. Electrical characterization of MFCs

Schematics of the experimental setup for electrical characterization of MFCs are shown in Fig. X1, and an in-depth description of the procedure was reported earlier (Randjelović et al., 2019). Since from the electrical point of view, a MFC can be considered as a non-ideal voltage source (Logan, 2009), this setup allows measurement of voltage and internal resistance in the MFCs. Briefly, based on preliminary research (Randjelović et al., 2019), resistors covering a wide range from 1 kΩ to 10 MΩ were chosen and placed on a protoboard SD35N (Velleman, Belgium). Then, the electrodes of each MFC were connected successively to each resistor on the protoboard to measure the established voltage in the MFCs. Voltage was read using a digital multimeter (Peak Tech 2025). For each measurement point, it was necessary to wait for the voltage to reach a quasi-steady-state when the voltage change was less than 0.5 mV/min (Capodaglio et al., 2013), which took 3 to 5 minutes on average, per resistor.

Knowing the voltage, resistor values, and Ohm's law allowed us to calculate the current flowing through each resistor and the developed power. Through Ohm's law, $V = I \times R$, the measured voltage was converted to current and power: $P = V \times I$. Afterwards, current (I_{den}) and power density (P_{den}) were obtained by dividing current and power by the total volume of the sediment. By implementing voltage-divider theory, peak power is expected at the external resistor having the closest value to the internal resistance of the MFC.

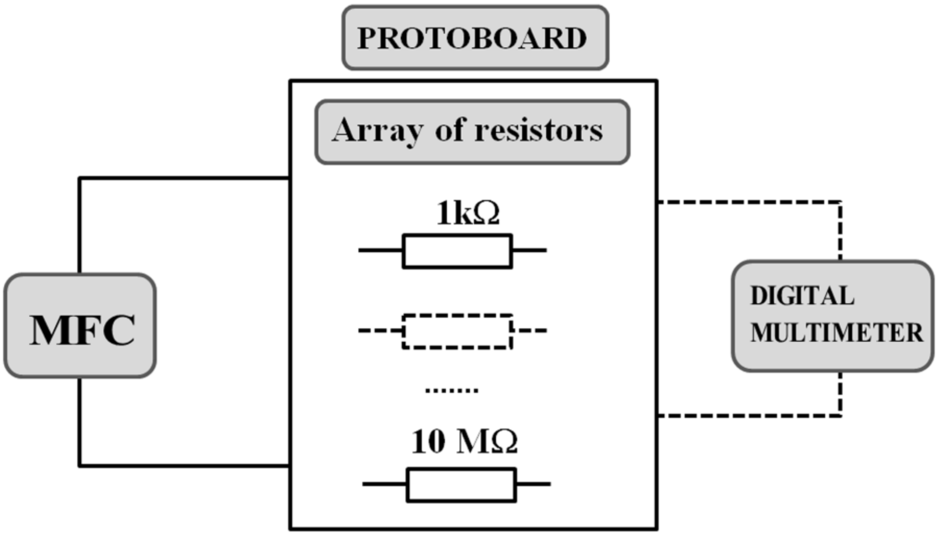

Fig. X1. Schematic experimental setup for electrical characterization of MFCs.[1]

2.3. Microbiological characterization

The composite sediment was analyzed immediately after it was collected to determine total numbers of specific groups of microorganisms. All determinations were conducted in triplicate by spread-plating appropriate serial dilutions (10^{-2}–10^{-10}) of a 1:9 homogenate of composite sediment onto agar plates that were incubated for 24 h for bacteria and 48 h for yeasts and moulds at 28 °C. Total chemoorganoheterotrophic bacteria (TC) were determined on nutrient agar (Torlak, Serbia), yeasts and moulds (YM) were determined on malt agar (Torlak, Serbia). Total anaerobic mesophilic chemoorganoheterotrophic (TAC) bacteria were determinate on nutrient agar supplemented with 0.5% glucose (Schaad and Forster, 1985), microorganisms able to utilize diesel (HD) as a carbon source were determined on mineral agar containing 2000 ppm of diesel oil (Beškoski et al., 2013). For determining the number of SRB, sulphite isolation agar was used (casein hydrolysate 10.0 g/L; Na_2SO_3, 0.5 g/L; Fe (II) citrate, 0.5 g/L).

2.4. Preparation of enrichment culture

To prepare enrichment culture of SRB, one gram of composite sediment was added to 100 mL of Baar’s medium (K_2HPO_4, 0.5 g/L; Na_2SO_4 4.5 g/L; NH_4Cl, 1 g/L; $CaCl_2 \cdot 4H_2O$, 0.1 g/L; $MgSO_4 \cdot 7H_2O$ 0.06 g/L; sodium lactate 6.0 g/L; yeast extract 1.0 g/L; $FeSO_4 \cdot 7H_2O$ 0.5 g/L; sodium citrate·$6H_2O$ 0.3 g/L (Zhang and Wang, 2017)) and incubated for 7 days at 28 °C under anaerobic conditions. Then, 1 ml of enrichment culture was added to another 100 ml of Baar’s broth and further enriched for another 7 days, and this procedure was repeated once more. To isolate pure cultures, enrichment cultures were suspended in saline solution and spread onto sulphite isolation agar. The plates were incubated under anaerobic conditions by use of the BD GASPAK^{TM} anaerobe container system, at 28 °C, until single black colonies were obtained (7 days). Pure cultures were obtained from selected black colonies by repeated inoculation in Baar’s broth.

2.4. Molecular characterization

Genomic DNA from isolated pure bacterial cultures was extracted using DNeasy Blood & Tissue Kit (Qiagen, Germany), according to the manufacturer’s protocol for bacterial cells. Primers used to amplify 16S RNA for PCR sequencing were 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-CGGCTACCTTGTTACGACTT-3′). Amplicons were extracted from 1% agarose gels and purified using the QIAquick PCR Purification Kit. Sequencing was performed by Macrogen service in the Netherlands. The sequences were checked for similarities to DNA sequences in the NCBI databases by using the BLAST programme.

3. Results and discussion

3.1. Chemical and microbiological characteristics of composite sediment

Composite sediment from the Danube River was characterized by a slightly alkaline pH, moderate organic carbon content and relatively high nitrogen content (Table 1). Regarding the contents of heavy metals, only Ni and Co concentrations in the composite sediment were higher than the upper limits specified by the Serbian Soil Quality Regulation (Official Gazette, 2019).

The total numbers of microorganisms from the physiologically different biochemical groups were in the 10^4-10^5 CFU/g range, indicating this river bed material could be a good microbial source for further research on MFCs (Table 1). In order to improve working conditions in the MFCs, it was necessary to increase the anaerobic processes, i.e., to stimulate the growth of SRB, to at least 10^7 CFU/g (Angelov et al., 2013).

Table 1. The initial composition of composite sediment used for MFC construction.

Content, unit	**Value**	**Content, unit**	**Value**	**Microbial content, unit**	**Value**
Water content, %	33.4	Hg, mg/kg	<0.01	TC, CFU/g	2.40×10^5
pH (1:2.5 in H_2O)	7.70	As, mg/kg	5.68	YM, CFU/g	7.90×10^3
$CaCO_3$, %	4.99	Co, mg/kg	12.1	TAC, CFU/g	2.40×10^4
C total, %	3.58	Ca, mg/kg	81066	HD, CFU/g	7.10×10^4
Organic C, %	2.98	Mg, mg/kg	15772	SRB, CFU/g	6.47×10^4
Inorganic C, %	0.60	Na, mg/kg	1150		
N total, %	4.74	K, mg/kg	4667		
S total, %	0.24	Fe, mg/kg	7715		
H total, %	0.89	Ni, mg/kg	51.8		
		Cu, mg/kg	10.4		
		Zn, mg/kg	93.7		
		Pb, mg/kg	18.3		
		Al, mg/kg	6849		
		Cd, mg/kg	0.31		
		Mn, mg/kg	287		
		Cr, mg/kg	52.3		
		Mo, mg/kg	<0.10		
		B, mg/kg	385		

3.2. Generation of voltage, current and power by MFC

The voltages for all four MFCs were measured daily as described above and afterwards, other parameters of interest were calculated. The maximum recorded voltages, current densities and power densities, per day, are listed in Table 2. It is obvious that the voltage increased with increasing resistance as shown in Fig. 1.

The control cell, MFC_0, generated a maximum voltage of 25.3 mV on the first day. The activity of MFC_I was measured for five days and reached its highest voltage of 261.2 mV on the second day. Similar results of about 200 mV and 300 mV were obtained by Abbas (Abbas et al., 2018) for MFC systems with sediment that had characteristics similar to our MFC_I The advantage of our river system is that this voltage is generated in a much shorter time, just two days, than their voltage, which was obtained only after 60 days. Organic compounds together with microorganisms in sediment play an important part in MFCs (Alipanahi and Rahimnejad, 2018). Without microorganisms, a small amount of voltage is generated, originating solely from organic matter, while in sediment originating directly from the river, there is a slightly larger voltage generation caused by the microbial community present therein utilizing the organic matter. The result of this difference in sediment composition is reflected in the 10-fold higher peak voltage produced by MFC_I compared to MFC_0.

Regarding MFC_{II}, it produced its maximum of about 480 mV on day 8. A very similar change of activity over time was observed for MFC_{III}, but it produced a higher peak of 520 mV. The sulphate and carbonates, substrates for microorganisms in the anode compartment of MFC_{II} and MFC_{III}, influenced voltage production. The difference in voltage between MFC_I, MFC_{II} and MFC_{III} is the obvious consequence of the presence of sulphates and carbonates. Li et al. (2019) also showed that by applying substrate in the form of sulphate, an increase in voltage production occurs. The maximum resulting voltage of 0.52 V, obtained with MFC_{III}, is close to the 0.54 V voltage achieved by Li (Li et al., 2019) in a MFC with a concentration of 1 mM sulphate.

Table 2.

Summary of peak voltage (V), current density (I_{den}) and power density (P_{den}) over time, for four types of MFCs: sterile sediment (MFC$_0$), regular sediment (MFC$_I$), one-month-old sediment with a substrates for SRB bacteria (MFC$_{II}$) and sediment with isolated bacteria (MFC$_{III}$). Resistance sweeps were performed over the range of 1 kΩ to 10 MΩ.

	MFC$_0$	MFC$_I$	MFC$_{II}$	MFC$_{III}$
U (mV)	25.3	261	480	521
I_{den} (mA/m^3)	0.93	145	241	192
P_{den} (mW/m^3)	0.004	4.25	10.6	8.80
days	day 1	day 2	day 8	day 10

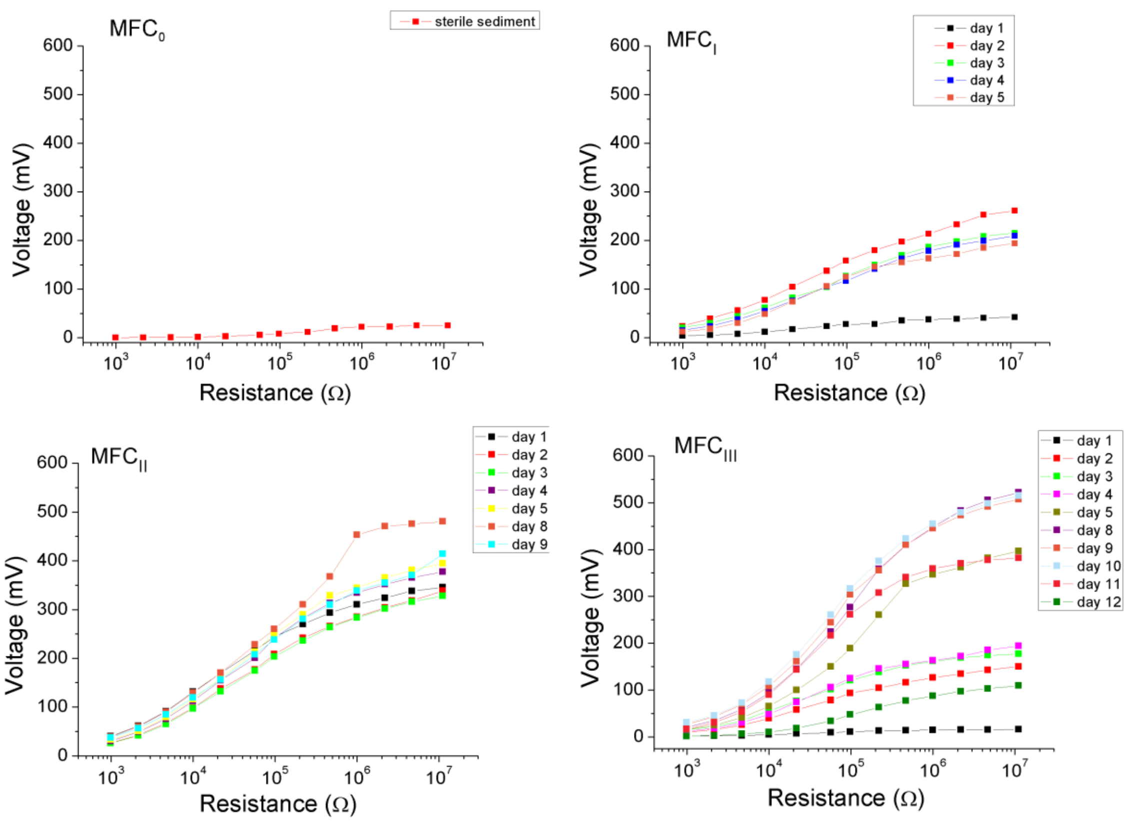

Fig. 1. Voltage output over a series of resistances overtime for four different types of MFCs: MFC$_0$, MFC$_I$, MFC$_{II}$ and MFC$_{III}$.[2]

The highest currents were obtained at the lowest resistor values used, as shown in Fig. 2. MFC$_0$ had very low current generation per cubic metre (m^3), 0.93 mA/m^3. This once again confirms the claim that without microorganisms there is no production of electricity, but rather a small amount comes from organic matter inherent in the sediment, so the potential difference between the two compartments with sterilized sediment leads to a small amount of voltage resulting in a small current (Abbas et al., 2019; Guo et. al., 2019). Days 2 and 3 were the best for the generation of current from MFC_I, i.e., 145.3 mA/m^3 (6.50 mA/m^2) and 130.3 mA/m^3 (5.90 mA/m^2), respectively. To the best of our knowledge, these amounts of current are by far the best results among the data reported in literature to date for the current generated by natural sediments. Reported currents from other natural sediments are 4.1 mA/m^2 (Nastro et al., 2019) and 0.75 mA/m^2 (Abbas et al., 2018). This makes the potential of Danube River sediment quite high, and potentially useful for energy generation. MFC_{II} generated good amounts of current, 251.8 mA/m^3 and 241.1 mA/m^3 on days 1 and 8, respectively. Substrate added to the MFC_{II} sediment positively influenced the amount of current generated relative to that generated by the river sediment with no additive in MFC_I. This result supports the hypothesis that MFCs could be more suitably applied to contaminated matrices (Li et. al., 2019). MFC_{III} produced its largest amounts of current on days 8 and 9, 192.9 mA/m^3 and 167.3 mA/m^3, respectively. MFC_{III} produced slightly less current than MFC_{II}. The result indicates that this could be caused by better degradation and electron release of the biostimulated microbial communities from MFC_{II} (Gojgić-Cvijović et. al., 2012; Xu, et. al., 2019). The three bacterial strains added to MFC_{III} did not produce increased amounts of current in comparison with MFC_{II}. Prasad and Tripathi (2018) reported natural sediment in 10 hybrid linked MFCs produced 870 mA/m^3 of current. The values of all parameters (current, voltage and power) can be adjusted depending on the applications, as shown in the literature (Prasad and Tripathi, 2018).

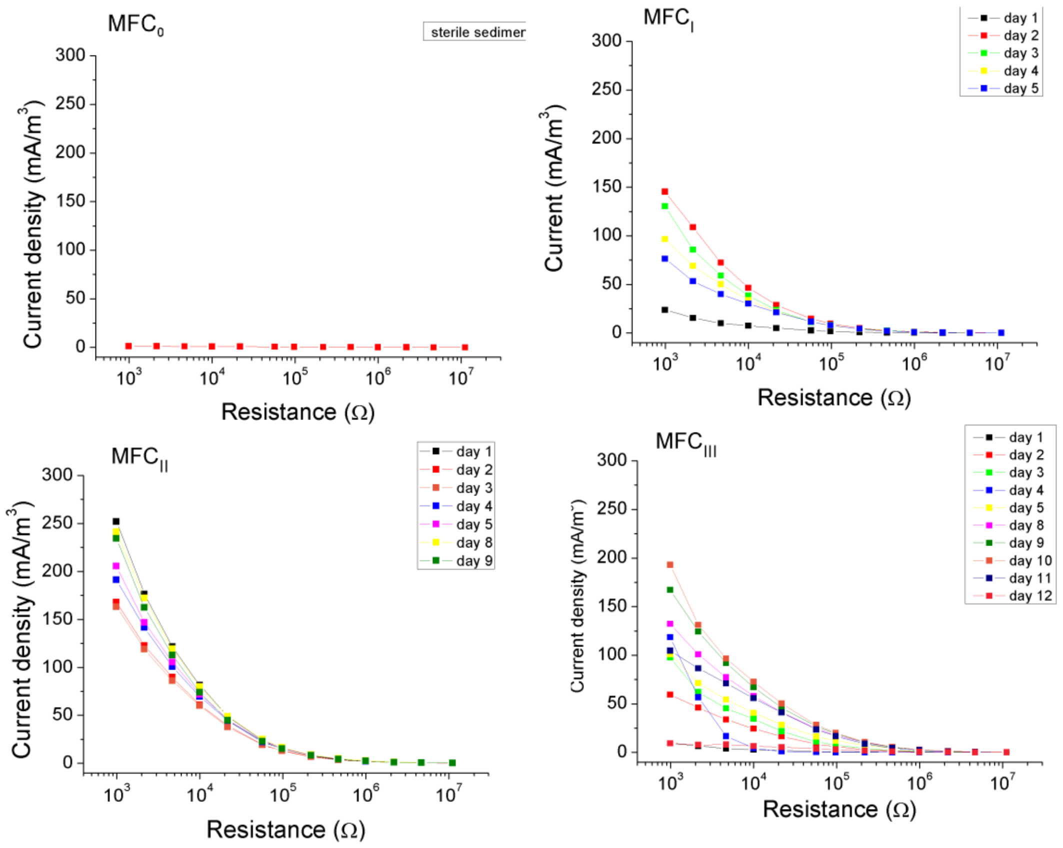

Fig. 2. Generated current density over a series of resistances as calculated from the measured voltages for MFC$_{0,}$ MFC$_{I}$, MFC$_{II}$ and MFC$_{III.}$[3]

The voltage obtained by successive measurements, and the calculated current over the chosen set of resistors were used to determine the power per cubic metre, and results are shown in Fig. 3. The power density was the lowest for MFC$_0$, 4.89x10^{-3} mW/m^3 with the 500 kΩ resistor. Somewhat greater power density (2.71 mW/m^3) was achieved by MFC$_I$ with the 2 kΩ resistor on day 2, and MFC$_{II}$ produced the highest power density in our study, 10.67 mW/m^3 (0.48 mW/m^2 with the 5 kΩ resistor), higher than 0.35 mW/m^2 reported earlier (Wang et al., 2019a). Interestingly, MFC$_{III}$ produced some of the lowest power densities we tracked; on day 10, it produced just 8.80 mW/m^3 power density with the 20 kΩ resistor.

In order to understand the relationship between power and current densities, we analyzed a polarisation curve for the data from our MFCs, Fig. 4. Fig. 3 shows the highest power density was obtained from MFC$_{II}$, and the activity was highest on day 8. Slightly less power density was obtained with MFC$_{III}$. Winfield (Winfield et. al., 2011) show an electrogenic community of microorganisms, with enough food sources (in the form of carbon, sulphur and carbonate), can produce power density of about 4 mW/m^2 as we obtained in MFC$_{II}$, giving power density of 10.6 mW/m^3. The small difference in power density between MFC$_{II}$ and MFC$_{III}$ comes from the difference in electron flow and solution conductivity (Logan, 2009). As Logan et al. (2019) shows, it has not been determined what has a bigger influence on higher power production – combinations of microbial cultures or specific isolated bacteria.

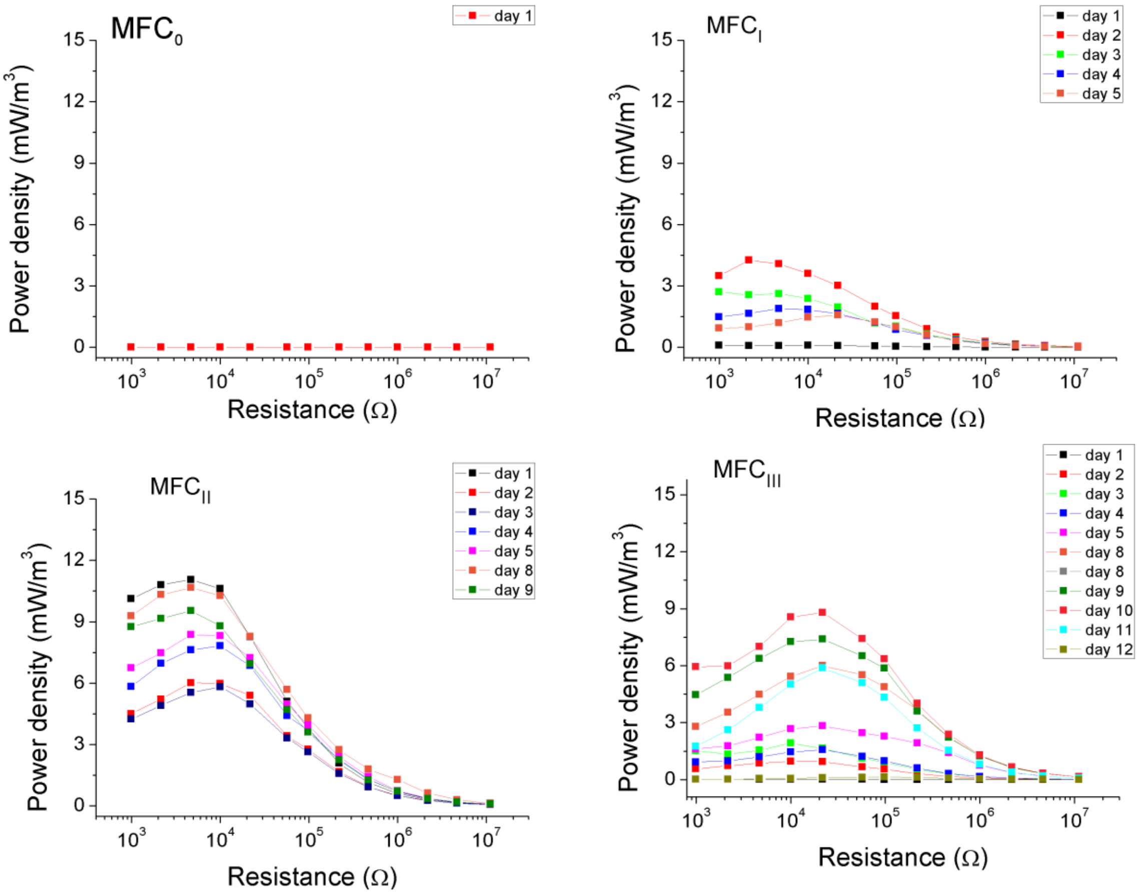

Fig.3. Power density per cubic metre over a series of resistances, for MFC$_{0}$, MFC$_{I}$, MFC$_{II}$ and MFC$_{III}$.[4]

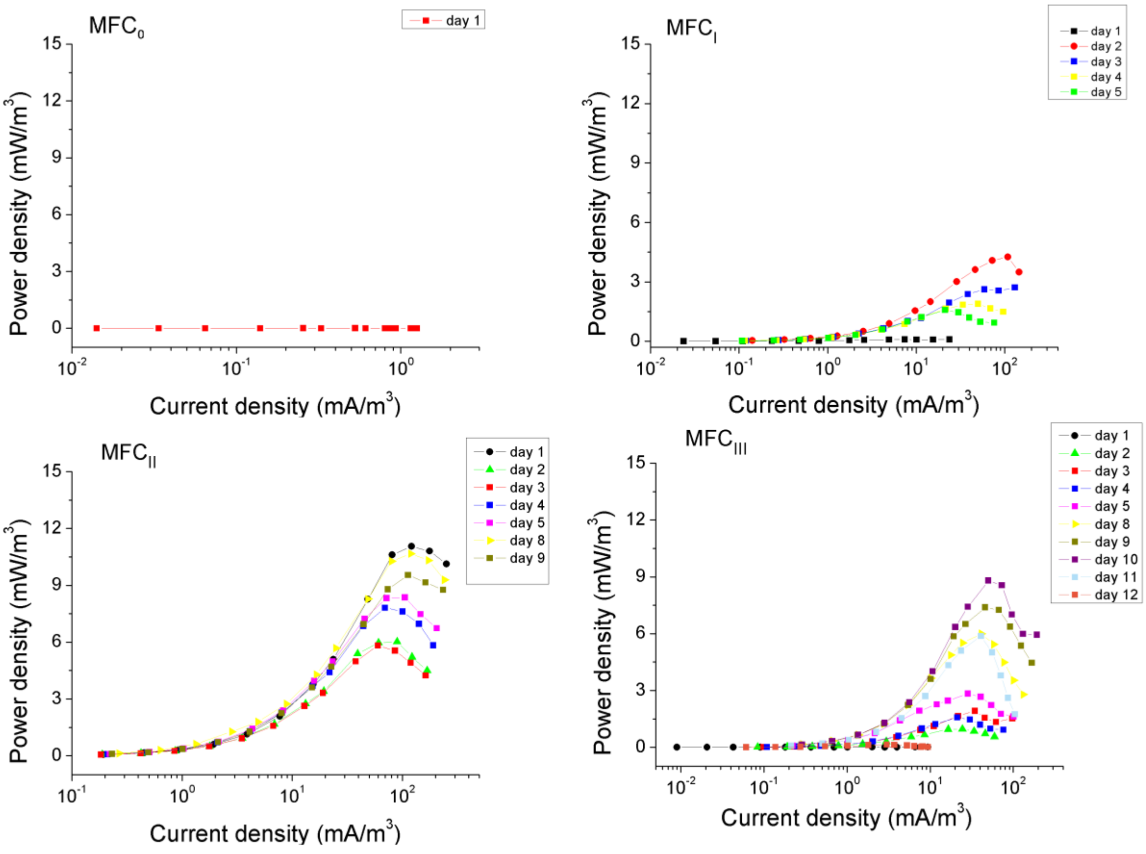

Fig 4. Power density per cubic metre versus current density over a series of resistances for MFC$_0$, MFC$_I$, MFC$_{II}$ and MFC$_{III}$.[5]

3.2. The mixture of individual bacterial species from the sediment used for MFCs

The composition of microbial species in the composite sediment was studied, with our greatest attention focused on anaerobic microorganisms. Pure cultures of anaerobic SRB bacteria were isolated from the composite sediment. 16S rRNA analysis showed three bacterial genera of this type predominated in the river sediment. These three genera were the Gram-positive endospore-forming bacteria, *Clostridium*, *Bacillus* and *Tepidibacter*. The predominance of these genera in the other described MFC systems was the reason we added individual pure cultures of these three genera to MFC$_{III}$ (Li et al., 2019; Xu et al., 2019; Li and Nealson, 2015). Based on the available literature, isolated strains of *Clostridium* (Abbas et al., 2019*)* *Bacillus* (Liu, et al., 2017) and *Tepidibacter* (Li and Nealson, 2015) can transfer extracellular electrons to the anode without the presence of a mediator (Guo et al., 2019). Among the isolated genera, *Clostridium* is the most widely described as the major producer of electricity and power in MFCs.

4. Conclusion

In this work, the potential of obtaining electrical current using Danube River sediment in an MFC was successfully demonstrated. The established presence of different groups of microorganisms from the river sediment successfully contributed to the operation of the MFC, by generating the highest voltage of 521 mV. A maximum voltage was obtained with sediment to which was added a mixture of three different SRB (*Clostridium*, *Bacillus* and *Tepidibacter*) that had been previously isolated from the composite sediment. The highest current density of 241.17 mW/m^3 was produced by one-month-old sediment with added substrates for SRB bacteria. The maximum power density calculated, 10.6 mW/m^3, was also recorded for this biostimulated composite sediment. It is noteworthy that free energy present in Danube River has shown a great potential for producing electricity by MFCs systems.

Thus, sediment MFCs are a natural source of free power and energy that could stimulate the degradation of organic pollutants that can be found in waters in small quantities. In order to expand their potential applications in environments, further studies are required to increase the current through parallel connection of more MFC systems like MFC_{III}.

5. Acknowledgments

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