Minimalistic soil microbial fuel cells for bioremediation of recalcitrant pollutants

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Abstract: Increased human, agricultural and industrial activities along with improper waste disposal leads to high levels of soil contamination and accumulation of recalcitrant contaminants in the environment. This global issue demands the use of green and sustainable technologies and soil microbial fuel cells (SMFC) can be a potential solution. We adopted minimalistic designs, based on low-cost carbon materials without any expensive catalyst and membrane, which makes the SMFCs suitable for in-field applications. We investigated the ability of the indigenous microbial population of the soil to use organic contaminants as the source of carbon and the enrichment of the electroactive consortium was monitored over time onto the electrode surface of the SMFCs. We tested performance in soil contaminated with pesticide and soil contaminated with hydrocarbons and compare the microbial enrichment process with respect to the case of non-contaminated soil.

1.Introduction

Increasing human, agricultural, and industrial activities cause widespread environmental pollution across the globe, which is a serious threat to the ecosystem as well as to human and animal health. Hazardous compounds, in the form of recalcitrant hydrocarbons, pesticides, heavy metals and micropollutants, contaminate soils/sediments, ground and surface waters. About 80% of the global wastewater is released into the environment without adequate treatment [1] and 400 million tons of hazardous waste is produced annually around the globe [2]. Although there are different physico-chemical techniques to deal with such pollutions, they are often complex, energy-consuming, and expensive. Despite the scientific advancements towards reducing these pollutants, there is still huge scope and need for developing low-cost, sustainable green technologies to address these environmental issues.

Minimising the risks associated with the accumulations of harmful chemicals in the environment is key to establish low-cost sustainable methodologies for the treatment of contaminated soil and water. To exploit the excellent capability of different microbial species to utilize recalcitrant compounds and converting them to harmless end products is crucial to achieve higher remediation efficiency. The efficacy of the microbes to oxidize organic contaminants as carbon source provides an understanding on microbial enrichment patterns in different kinds of soils and can help to develop innovative techniques for bioremediation. Conventional bioremediation techniques rely on either biostimulation (addition of nutrients or O_2) or bioaugmentation (addition of microorganisms) [3, 4]. These approaches have many limitations, which include limited water solubility and high chemical reactivity of oxygen with the minerals in soil and subsurface environment. Bioaugmentation can also be ineffective because of incompatibility with the indigenous microorganisms and lack of effective electron donors/acceptors. Most contaminated sites in soil and subsurface environment are anoxic and hence relying on the activities of indigenous microorganisms is often more practical [5].

By introducing microbial fuel cell (MFC) strategy as a remediation technique, we can effectively exploit the ability of electroactive bacteria to degrade organic contaminants and simultaneously generate bioelectricity. The protons generated in the microbial oxidation, travel to the cathode and the electrons flow through the external

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circuit to reach the air cathode, where a terminal electron acceptor (Oxygen) is reduced, and the overall cell potential is measured. Several studies have demonstrated the ability of MFCs to degrade recalcitrant pollutants like hydrocarbons and pesticides [6, 7], thus suggesting the technology as a promising green strategy to promote advanced environmental remediation. In this study, we test the use of low-cost air-cathode soil microbial fuel cells (SMFCs) for the degradation of recalcitrant pollutants in soil. We have tested the electrochemical performance of the SMFCs in soil contaminated with the pesticide atrazine and soil contaminated with petroleum hydrocarbon, along with the pollutant degradation ability of the system. The simple design implemented for the SMFC, with no use of catalyst and no membrane, facilitates the scale-up of the technology for field tests and guarantee its cost-effectiveness.

2. Materials and Methods

2.1. Materials

The chemicals used in this experiment were purchased from Alfa Aesar/Thermo Fischer Scientific (Lancashire, UK) and Sigma-Aldrich (Merck Life Science UK Limited, Gillingham, UK). The graphite felt is purchased from Online Furnace Services Ltd (Scotland, UK), screws were procured from Bluemay Limited (Wiltshire, UK) Titanium wire (diameter 0.25 mm) was obtained from VWR International Ltd. (Leicestershire, UK). Non-contaminated soil was collected around the campus area of the University of Bath from a maximum depth of 30 cm below the surface. The soil used for pesticide study was purchased from Homebase (https://www.homebase.co.uk/). The soil used for hydrocarbon study was provided by Acciona (https://www.acciona.com/) and collected from a contaminated area in a Machinery Park in Noblejas (Toledo, Spain). The contaminated area can be classified as surface spill from a drilling machine with the oil infiltrated about 20-25 cm deep. The soil was sieved at 0-2 mm after excavation to remove stones and gravels. The non-contaminated soil, collected from the university of Bath campus, was also cleaned of visible stones, gravels, roots and leaves before use. The hydrocarbon contaminated soil was mixed with surfactant Tween-80 at 0.2 mass% [8] to enhance the bioavailability. The physicochemical properties of the three types of soils are summarised in Table 1. The percentage of organic matter is higher in non-contaminated soil, but the amount of phosphorus and potassium is higher in the soil contaminated with hydrocarbons. In addition, concentration of TPH (Total Petroleum Hydrocarbon) is very high in the contaminated soil.

Table 1: Physicochemical properties of the soils used in this study

Parameters	**Non-contaminated soil [9]**	**Soil contaminated with Pesticide**	**Soil contaminated with Hydrocarbons**
pH	6.5	7.4 ± 0.18	7.9±0.05
Moisture content	53%	32%	20%
Nitrogen	<0.001%	<1%	<1%
Phosphorus	<10 mg/kg	<10 mg/kg	70.7 mg/kg
Potassium	<150 mg/kg	-	1800 mg/kg
Organic matter	17.4%	7.27%	10.9%
Concentration of contaminant	N/A	5mg/kg (Atrazine)	34000 mg/kg (Total Petroleum Hydrocarbon)

2.2. SMFC Design and Operation

SMFCs for three experimental conditions were operated according to the type of soil (non -contaminated, with pesticide and with hydrocarbon), as shown in Figure 1. The SMFCs were constructed in triplicates and fitted in small polypropylene boxes (dimension: 12 cm^2 X 12 cm h), which were insulated with aluminium foil to avoid photolysis of the pesticide and hydrocarbons. The boxes were further loosely covered with lids to avoid evaporation. Graphite felt electrodes were used for both the anode and the cathode (dimension: 8 x 8 x 0.6 cm for SMFCs with non-contaminated soil and pesticides and 7 x 7 x 0.4 cm for the SMFCs with hydrocarbon contaminated soil) and kept at a fixed distance of 4 cm with nylon screws. The anodes were pre-treated to increase the hydrophilicity and the roughness of the carbon nanofibers, as previously described [10]. No external catalyst was used for the cathode. The anodes were buried in the soil and the cathodes were exposed to air. The soil itself was used as a separator for the two electrodes, while the natural stratification in soils ensures negligible oxygen transfer to the anode. The electrodes were connected to an external resistance of 500 Ω and to a data acquisition system (DAQ6510, Keithley instruments, Tektronix UK Ltd.) to monitor the output voltage over time. The current was calculated using Ohm's law $V=IR$ and was normalized to the surface area of the electrodes. The soil was kept moistened with tap water. Titanium wire was used for connecting the electrodes.

Hydrocarbon fractions from the SMFCs were analysed using Gas chromatography-mass spectrometry (GC-MS) and were performed through an external analytical company (Eurofins Chemtest Ltd, Cambridge, UK). The pesticide was collected from homogenized soil and analysed using high-performance liquid chromatography coupled with tandem mass spectrometry.

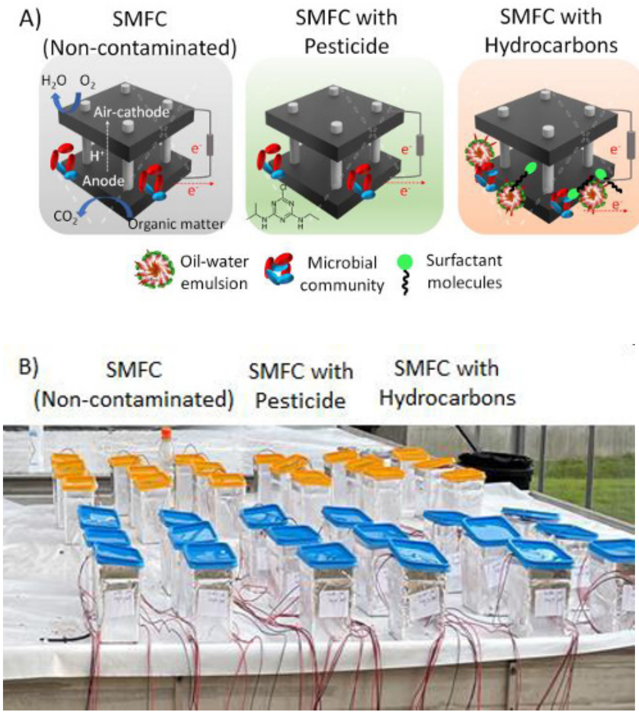

Fig. 1. A) Schematic of the SMFCs working principles and operation in different soils. B) Experimental set-up

3. Results and Discussion

The performance of the SMFCs in soils with different pollutants was investigated. The model pesticide tested was atrazine, which was used to spike non-contaminated soil. Atrazine is a herbicide widely used to control weeds. It is sprayed on row crops and sometimes also on residential lawns and highway/railroad rights-of-way [11]. Atrazine can enter water supplies from the soil and its contamination in public/private water supplies above the drinking water standards (maximum contaminant level for drinking water: 0.003 mg/L) set by United States Environmental Protection Agency-EPA raises concern as it has been linked to adverse reproductive effects in amphibians and other wildlife and reduce primary production in aquatic communities by inhibiting photosynthesis. Currently it is also being studied as a potential carcinogen to both aquatic and human life. The other soil tested was contaminated with petroleum hydrocarbons. Petrochemical contamination due to oil spills is a major concern because of its toxicity and recalcitrance. Oil companies produce billions of gallons of salty and toxic wastewater out of the oil wells and if not stored properly until treated further, there is a potential risk of spill which contaminates surface water including vegetation and drinking water [12]. The clean-up standard for TPH in soil varies on different factors, including the types of hydrocarbon present, depth of groundwater, proximity of human population and the future use of the site etc. Despite these variables, the most commonly used soil cleanup standard for TPH is 100 mg/kg [13].

The performance of the SMFCs in these two types of soils was compared with the case of non-contaminated soil. Figure 2 shows the current density generated by the SMFCs in the different soils over time. As shown, in non-contaminated soil, the enrichment of the microbial consortium at the anode occurred faster than the soil contaminated with pesticide and hydrocarbons. The initial lag-phase (with voltage below 0.05 V, current density <16 mA/m^2) lasted approx. 10 days in non-contaminated soil, while, for the case of soil with pesticides, the lag phase lasted >13 days. In the case of soil contaminated with hydrocarbons, however, the average current density remained at about 25 mA/m^2 (~ 0.06 V) for the entire duration, suggesting the microbes did not attain the state of enrichment. In the case of non-contaminated soil, an average steady output voltage of 0.204 ± 0.068V (Corresponding current density 64 ± 21 mA/m^2) was obtained, after reaching a value of 0.18 V (56 mA/m^2), while the output current density in the case of SMFC in pesticide contaminated soil decreased until a value of 34 mA/m^2 (~0.11 V) on day 19. The pesticide SMFC started to show improvements from 22nd day onwards (~ 28 mA/m^2, 0.09 V) and steadily reached to (~53 mA/m^2 ,0.17 V) in about 30 days, suggesting that this is the actual exponential phase for the microbial consortium in the SMFC in soil contaminated with pesticide. The abrupt rise and fall in the potential for the SMFC in soil contaminated with pesticide may be due to some alteration in the microbial consortium or to variation in the operational conditions (such as temperature and water content in soil).

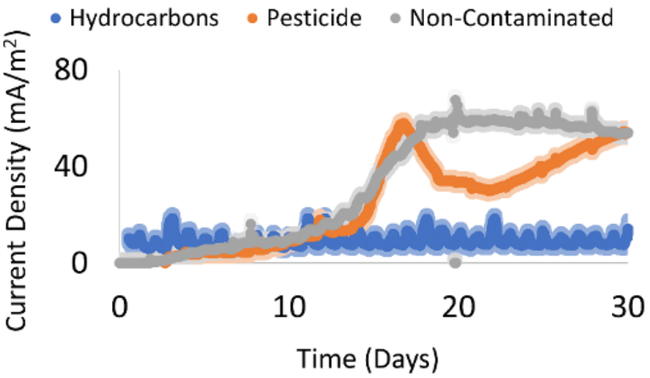

Fig. 2. Current density showing enrichment patterns over time for three SMFCs. Data is the average from three replicates. Shades show the standard error of replicates

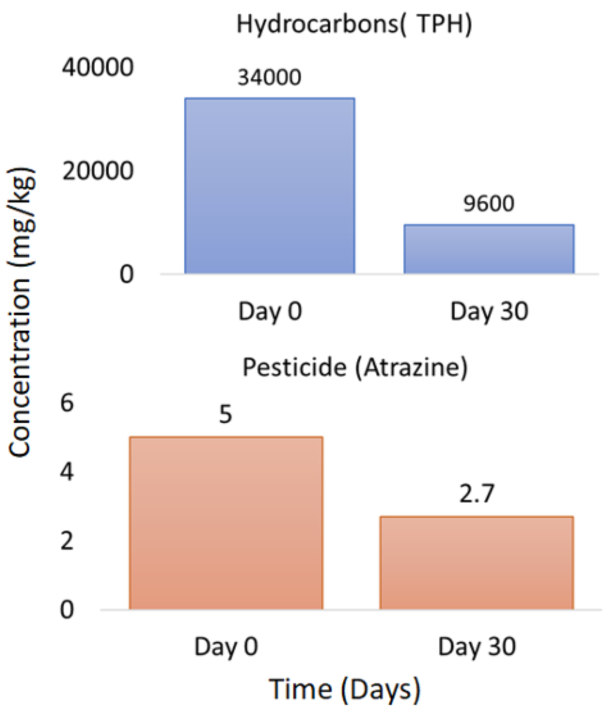

Fig. 3. Degradation pattern of Total Petroleum Hydrocarbon (TPH) and pesticide (Atrazine) in SMFCs over a period of 30 days

The poor performance of the SMFC in the soil contaminated with very high concentrations of hydrocarbons demonstrates that its toxicity prevents the development of an electroactive biofilm at the anode. No bioaugmentation (addition of enriched microbial consortium externally) strategy is adopted in this study, and the SMFC performance is uniquely dependent on the indigenous microbial consortium. It is likely that the formation of electroactive biofilm is hindered in presence of such high concentrations of hydrocarbon fractions, leading to lower output potential.

Figure 3 shows the degradation of atrazine and TPH obtained with the SMFCs. As shown, in the case of soil contaminated with atrazine, a degradation of about 46% was observed after 30 days. In the case of hydrocarbons, a much better degradation in the TPH was observed, reaching a value of about 72% on day 30. Although future investigation is required to assess the impact of bioelectrochemical routes in atrazine degradation, our results suggest that hydrocarbon degradation is achieved by other biochemical pathways. Further research must necessarily investigate the effect of different types of hydrocarbons and lower concentrations, as well as the relevance of biostimulation and bioaugmentation in the process.

4. Conclusions

There is a clear need for development of low-cost and easy-to-scale-up strategies for the bioremediation of recalcitrant pollutants in soils. Soil microbial fuel cells can be the solution, considering key features such as self-powered operations and minimum maintenance requirements. In this study, a cost-effective SMFC design was implanted and its performance in different types of soil, was investigated for the first time. Indigenous microbial population can significantly degrade organic matter of natural soil and it can also be used for the removal of contaminants such as atrazine. However, the concentration of the contaminants has crucial role in attaining microbial enrichment. Very high concentration of contaminant, as in the case of soil contaminated with hydrocarbons, can markedly limit the generation of high output voltages. Abundance of different microbial consortium plays an important role in degradation of pollutants and generation of electricity. Therefore, molecular analysis of the microbial consortium can be considered in future for more in-depth understanding.

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References

1. United Nations, Sustainable Development Goals, *https://www.un.org/en/sections/issues-depth/water/*, accessed online on October 26, 2021

2. UNEP. UN Environment. Available online: *https://web.unep.org/,* accessed online on October 26, 2021

3. M. Tyagi, M.M.R. da Fonseca, C.C. de Carvalho, *Biodegradation*, **22**, 231-241, (2011)

4. G.O. Adams, P.T. Fufeyin, S.E. Okoro, I. Ehinomen, *Int. J. Environ. Biodeterior. Biodeg*. **3**, 28-39, (2015)

5. M. Sharma, A. Nandy, N. Taylor, S.V. Venkatesan, V.O. Kollath, K. Karan, V. Thangadurai, N. Tsesmetzis, L. M. Gieg, J Hazard Mater. **389**, 121845, (2020)

6. G. Mohanakrishna, R.I. Al-Raoush, I. M. Abu-Reesh, D. Pant, RSC Adv, **9**, 41409-41418, (2019)

7. X. Cao, H.L. Song, C.Y. Yu, X.N. Li, Bioresour Technol, **189**, 87-93, (2015)

8. 3. C.C. Lai, Y.C. Huang, Y.H. Wei, J.S. Chang, J Hazard Mater. **167**, 609-614, (2009)

9. J. Dziegielowski, B. Metcalfe, P. Villegas-Guzman, C.A. Martínez-Huitle, A. Gorayeb, J. Wenk, M. Di Lorenzo, Appl Energy **278**, 115680, (2020)

10. S. Monasterio, M. Di Lorenzo, Chem Eng Sci, **198**, 108-116, (2019)

11. United States Environmental Protection Agency (EPA*), https://www.epa.gov/national-aquatic-resource-surveys/indicators-atrazine*, accessed online on October 29, (2021)

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12. A. Nandy, J.R. Radović, B. Novotnik, M. Sharma, S.R. Larter, V. Thangadurai, Bioresource Technol Reports, **11**, 100449, (2020)

13. T.C. Michelsena, C.P. Boyce, J. Soil Contam, **2**, 1-16, (1993)