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Enhanced removal of petroleum hydrocarbons using a bioelectrochemical remediation system with pre-cultured anodes

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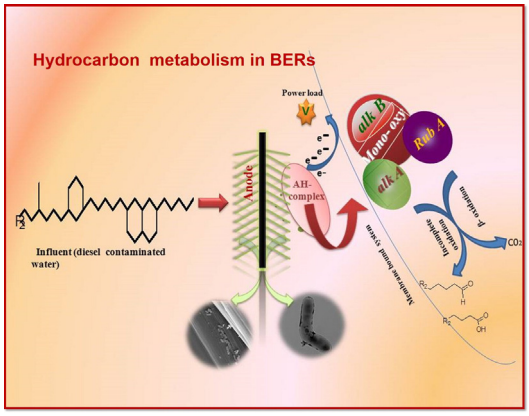
GRAPHICAL ABSTRACT



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ABSTRACT

Bioelectrochemical remediation (BER) systems such as microbial fuel cells (MFCs) have recently emerged as a green technology for the effective remediation of petroleum hydrocarbon contaminants (PH) coupled with simultaneous energy recovery. Recent research has shown that biofilms previously enriched for substrate degrading bacteria resulted in excellent performance in terms of substrate removal and electricity generation but the effects on hydrocarbon contaminant degradation were not examined. Here we investigate the differences between enriched biofilm anodes and freshly inoculated new anodes in diesel fed single chamber mediatorless microbial fuel cells (DMFC) using various techniques for the enhancement of PH contaminant remediation with concomitant electricity generation. An anodophilic microbial consortium previously selected for over a year through continuous culturing with a diesel concentration of about 800 mg l^{-1} and which now showed complete removal of this concentration of diesel within 30 days was compared to that of a freshly inoculated new anode MFC (showing 83.4% removal of diesel) with a simultaneous power generation of 90.81 mW/m^2 and

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15.04 mW/m^2 respectively. The behaviour of pre-cultured anodes at a higher concentration of PH (8000 mg l^{-1}) was also investigated. Scanning electron microscopy observation revealed a thick biofilm covering the pre-cultured anodic electrode but not the anode from the freshly inoculated MFC. High resolution imaging showed the presence of thin 60 nm diametre pilus-like projections emanating from the cells. Anodic microbial community profiling confirmed that the selection for diesel degrading exoelectrogenic bacteria had occurred. Identification of a biodegradative gene (*alk*B) provided strong evidence of the catabolic pathway used for diesel degradation in the DMFCs.

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1. Introduction

Soil and groundwater contamination with petroleum hydrocarbon (PH) has long been a serious environmental and public health concern. Massive consumption of these compounds leading to spillages, and leakages from underground storage tanks, are the two dominant sources of groundwater contamination. Of these petroleum hydrocarbon contaminants, diesel range hydrocarbons (DRH) have been documented as one of the most abundant pollutants; they can be biodegradable in both oxic and anoxic conditions (Huang et al., 2011). Microbial removal of these DRH compounds is claimed to be an efficient, economic, versatile alternative to the established physicochemical treatments, which are prone to causing recontamination with secondary contaminants (Hong et al., 2005). While such bioremediation techniques can work well, they may involve high energy inputs and lead to the production of sludge. In addition, this process is very slow especially in underground anaerobic conditions where nutrients are limited (Morris et al., 2009).

A bioelectrochemical technology, Microbial Fuel Cells (MFC), shows potential as an effective approach that can exploit microorganisms for treating recalcitrant compounds while generating electricity in the process. This technology has recently been proposed for the remediation of petroleum hydrocarbon contaminants by capitalising on the biocatalytic potential of anodophilic bacteria (Morris et al., 2009). These anode respiring bacteria growing on an anaerobic anode simultaneously reduce the anode and oxidise substrates such as hydrocarbons and other organic substances present in waste water. Until now, only a few researchers have explored the bioelectrochemical remediation (BER) of petroleum hydrocarbon contamination through MFC technology (Morris et al. 2009; Mohan and Chandrasekhar, 2011; Wang et al., 2012, Lu et al., 2014). Mohan and Chandrasekhar (2011) documented a more effective degradation of field petroleum sludge utilising MFC technology when compared with a conventional anaerobic treatment. A recent publication (Morris and Jin, 2012) highlighted the possibility of using sediment microbial fuel cells for hydrocarbon contaminated sites. Investigations from Wang et al. (2012) also provided solid evidence for the *in situ* bioremediation of hydrocarbons using a BER system.

Recently, electrodes colonised with mixed consortia have received much attention owing to their stability and performance in terms of degradation of contaminants and electricity generation (Holmes et al., 2004; Ki et al., 2008). Compared to pure cultures, enriched communities are more stable and robust due to their interactions in biofilms, interspecies electron transfer mechanisms and adaptability. Several reports have documented considerable diversity in these communities which indicates possibility that larger communities are able to be sustained in MFC systems even after multiple transfers (Kim et al., 2006; Logan and Regan, 2006). A survey of the existing literature divulged a steady trend in the community organizations of pre-cultured MFC leading to a single dominant group (Yates et al., 2012). On the other hand, many reports have been published in which gamma-proteobacteria were predominant in wastewater enriched MFC systems, whereas similar MFC systems have instead resulted in phenotypically uncharacterised communities (Kim et al., 2006; Morris and Jin, 2008).

These inconsistencies in the reported data suggest that, firstly, our understanding of the ecology of exoelectrogens (electrochemically active bacteria), and secondly, the effects of selection on microbial communities at a cellular level in relation to the performance of the system, remain inadequate. One of the major aims of our group is to develop and study a laboratory scale diesel-fed mediator less microbial fuel cell (DMFC) with an emphasis on the interaction between microbe-anode-organic contaminant in MFC systems. While this has been examined in earlier versions of MFCs with different substrates, it is difficult to compare our results even with the existing petroleum hydrocarbon-fed MFC reports (Mohan and Chandrasekhar, 2011; Morris and Jin, 2008; Wang et al., 2011). These had focused on the possibilities of utilising MFCs for PH degradation using different types of MFC configurations and inoculums. To the best of our knowledge, no MFC studies have addressed the effects of pre-enrichment of the anode community, through previous long-term culture, on contaminant removal, power generation and the prevalence of catabolic genes. In this study, we conducted various experiments to examine power production and the bioelectrochemical degradation of DRH contaminants from DMFCs. Furthermore, the performance of a previously enriched anode community has been compared to that of a freshly inoculated anode and analysed at a molecular level using community profiling and the determination of the diversity of catabolic gene (*alk*B) sequences present in our DMFC reactors.

2. Materials and methods

2.1. Diesel-fed mediatorless MFC

Single cell MFC systems were constructed from laboratory bottles (320 ml capacity, Schott) as previously described (Logan, 2008) with a modification to increase electrode area. The anode electrodes contained carbon fibre brushes with two wire titanium cores that had an initial surface area of 6.99 m^2 g^{-1}. The anodes were cleaned by soaking overnight with acetone followed by pre-treatment with sulfuric acid (concentrated, 100 ml l^{-1}) and heat treatment to improve the geometric surface area of the brush as described by Feng et al. (2010). The cathode was made using flexible carbon cloth coated with a hydrophobic PTFE layer (Cheng et al., 2006) with added diffusional layers on the air breathing side to cut down fouling rate and evaporation of hydrocarbons, whereas the hydrophilic side was coated using a mixture of nafion perfluorinated ion exchange ionomer binder solution, carbon, and platinum catalyst (0.5 g of 10% loading). DMFCs were operated in fed batch mode until the voltage fell to a low level (≤10 mV), and then the growth solution was replaced under anaerobic chamber (10% hydrogen, 10% carbon dioxide and 80% nitrogen) (Don Whitley Scientific, MG500, Australia) conditions. After the complete acclimation of the system to the different concentrations of substrates, repeatable cycles of current were generated. The electrodes were connected using copper wire with all exposed metal surfaces sealed with a non-conductive epoxy resin (Jay Car, Australia).

2.2. MFC inoculation and operation

Effluent originating from anaerobic and aerobic sludge, and hydrocarbon contaminated groundwater samples served as the microbial anodic inoculum. DMFCs were operated in a fed batch mode using an anolyte medium (g l^{-1}) consisting of Na_2HPO_4 4.09, NaH_2PO_4 2.54, NH_4Cl 0.31, KCl 0.13, trace elements and vitamins (Oh et al., 2004).

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