Chemical Engineering Journal 332 (2018) 647–657

Contents lists available at ScienceDirect

Chemical Engineering Journal

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

Microbial community and bioelectrochemical activities in MFC for degrading phenol and producing electricity: Microbial consortia could make differences

Huzairy Hassan[a,b], Bo Jin[a,*], Erica Donner[c], Sotirios Vasileiadis[c], Christopher Saint[c], Sheng Dai[a,*]

[a] School of Chemical Engineering, The University of Adelaide, Adelaide, SA 5005, Australia
[b] School of Bioprocess Engineering, Universiti Malaysia Perlis, Kompleks Pusat Pengajian Jejawi 3, Arau 02600, Perlis, Malaysia
[c] Future Industries Institute, University of South Australia, Mawson Lakes, SA 5095, Australia

ARTICLE INFO

Keywords:
Microbial consortia
Microbial fuel cell
2,4-Dichlorophenol
Arcobacter
Bacillus
Cloacibacterium

ABSTRACT

Microbial fuel cell (MFC) and its reactor systems have been intensively investigated for energy production and wastewater treatment using wild bacterial consortia. Yet, there is lack of detailed studies on understanding how multiple wild microorganisms could alter bioelectrochemical reactions in MFC for degrading toxic organic contaminants and generating electricity from industrial wastewater. Hence, this study evaluates the microbial community and bioelectrochemical activities in a lab-scale MFC reactor inoculated by petrochemical industrial microbial consortium (IMC) and domestic microbial consortium (DMC), aiming to enhance MFC performance for degrading 2,4-dichlorophenol (2,4-DCP) and producing electricity. Cutting-edge microbiology analysis techniques were used for identifying microbial community in suspension and biofilm in the IMC and DMC inoculated MFC systems. Research focused on evaluating how the variable microbial population and 2,4-DCP feeding could affect bioelectrochemical activities and MFC performance. *Arcobacter*, *Aeromonas*, *Pseudomonas*, *Acinetobacter*, *Cloacibacterium*, and *Shewanella* sp. in DMC were found to be important bacteria for 2,4-DCP degradation while *Bacillus* sp. dominated IMC contributing to higher electricity production. This would be the first discovery of *Cloacibacterium* sp. contributing phenol degradation in MFC. IMC-MFC reactor performed well in producing high 156 mA/m^2 current density with 41% phenolic degradation, while DMC-MFC showed promising 62% 2,4-DCP reduction and 123 mA/m^2 current production. MFC systems performed highly comparable 2,4-DCP degradation efficiency than the conventional anaerobic biodegradation. These results demonstrate that wastewater-inoculated MFCs can be capable of simultaneous energy generation and phenolic degradation and provide new insights that may assist with future MFC optimisation.

1. Introduction

Microbial fuel cells (MFCs) have emerged as a promising technology for sustainable energy production and wastewater treatment, such as reduction of chemical oxygen demand (COD). Current research mainly focuses on increasing the amount of electricity generated by MFCs which use exoelectrogenic bacteria to produce energy with simultaneous biodegradation of organic contaminants present in the wastewater. Due to the increasing environmental impacts of wastewater from industrial activities, MFC are viewed as an important and sustainable technology for wastewater treatment. The MFC technology offers a number of advantages, including the direct production of energy and value-added products, reliable wastewater treatment performance through effective combination of biological and electrochemical processes, and operating stability to be achieved through real-time monitoring and control [1]. MFCs may produce up to 1.43 kWh/m^3 from a primary sludge or 1.8 kWh/m^3 from a treated effluent [2]. On average, MFCs consume only 0.024 kW or 0.076 kWh/kg-COD, which is about ten times less than activated sludge bioprocesses (~0.3 kW or 0.6 kWh/kg-COD) [3]. This suggests that MFCs operate at a very efficient energy consumption level compared to conventional wastewater treatment whilst also recovering energy via exoelectrogenic-based bioelectrochemical processes. Furthermore, a much smaller amount of sludge is produced (0.07–0.16 g VSS/g COD cell yield) in an MFC than in an activated sludge process (0.35–0.45 g VSS/g COD) [4]. This is significant given that sludge management is a considerable problem in industrial wastewater treatment.

The latest research findings demonstrate promising systematic

* Corresponding authors.
E-mail addresses: bo.jin@adelaide.edu.au (B. Jin), s.dai@adelaide.edu.au (S. Dai).

http://dx.doi.org/10.1016/j.cej.2017.09.114
Received 19 June 2017; Received in revised form 19 September 2017; Accepted 19 September 2017
Available online 20 September 2017
1385-8947/

outcomes in electricity generation and wastewater treatment using MFCs. For instance, noble materials (Acrylonitrile Butadiene Styrene and Nanocure) were found to be excellent as MFC electrodes to enhance electricity generation (~4.5 mW/m^2) and COD reduction (~53%) [5]. Graphene oxide supported magnesium oxide catalytic electrode improved power density of 756 mW/m^2 and COD reduction of 80% [6]. An up-flow MFC system has been designed to achieve maximum power generation (372 mW/m^2) with very high cell density retention [7]. Enhanced power generation (1063 mW/m^2) were reported for a lab-scale MFC system using a flame-oxidized hematite anode catalyzed by anodic dominant *Geobacter* sp. [8]. However, the relatively expensive materials and high-profile configuration make the practical applicability of these MFC designs for large –scale processes somewhat debatable.

The aforementioned studies typically evaluated the MFC performance for electricity generation and COD reduction. By contrast, there are still only limited studies reported in the literature which systematically investigated the ability of MFC systems to remove specific toxic pollutants such as phenolic compounds and other hazardous chemicals [9]. There are several reported studies on investigating the removal of phenols and its derivatives by bacterial strains or mixed culture from wastewater sludge in MFC systems [10–12]. Phenolic compounds are major toxic contaminants that are present in many industrial wastewaters, such as those derived from chemical, pharmaceutical, textile and oil refinery industries [13]. These substances are highly recalcitrant and of concern for their toxicity, suspected carcinogenicity and mutagenicity [14,15]. They are therefore the focus of considerable research.

Aiming to develop a MFC system for removing 2,4-dichlorophenol (2,4-DCP) and producing electricity from industrial wastewater, this study analysed microbial community and bioelectrochemical reactions in a double chambered MFC reactor which was inoculated by petrochemical industrial microbial consortium (IMC) and domestic microbial consortium (DMC). The systematic investigations of the natural consortia-inoculated MFC were conducted to better understand bioelectrochemical activities for enhancing MFC performance. Research focused on evaluating how the variable microbial population and phenol feeding could affect bioelectrochemical activities and MFC performance. 2,4-DCP as a phenol derivative is considered as refractory hazardous pollutant in many industrial wastewaters. Special attention was given to determining changes in both of these MFC microbial communities in response to the addition of 2,4-DCP. The microbial communities were analysed using partial 16S rRNA Illumina HiSeq sequencing and amplicon screening [16]. The dominant operational taxonomic units (OTUs) identified in these MFC systems and their phylogenetic affiliations were explored. In addition to promoting a practical and sustainable recalcitrant pollutant treatment in the MFC, this study also provides mainstays in microbial behaviour exploration for systematically selecting the suitable microorganisms profoundly involved in phenolic degradation as well as MFC electron transfer activities.

2. Materials and methods

2.1. Mixed bacterial consortia and growth media

2.1.1. Mixed bacterial consortia

The domestic microbial consortium (DMC) was derived from domestic wastewater collected from Glenelg Wastewater Treatment Plant, South Australia. The industrial microbial consortium (IMC) was derived from petrochemical wastewater collected from Australia Mobil Oil Plant, South Australia. Both samples were collected from clarifier tanks in the form of mixed sludge. The DMC sample was used directly as an inoculum for MFC experiments. The IMC sample was first separated using a separating funnel and the remaining sludge was used as the inoculum. MFCs were inoculated with either 30% (v/v) of DMC- or IMC-derived inoculum. The concentration of total phenolic compound in both domestic and industrial wastewaters was determined according to Garcia et al. [17]. Wastewater quality data for both wastewaters are provided in Table S1 (Supplementary Data).

2.1.2. Growth media

The growth medium for both microbial consortia was artificial wastewater consisting of 2.0% glucose, 0.386 g/L $(NH_4)_2SO_4$, 0.149 g/L K_2SO_4, 3.31 g/L NaH_2PO_4, 10.31 g/L Na_2HPO_4, 1 g/L NaCl, 0.2 g/L $MgSO_4$ and 12.5 mg/L vitamins (K and B_2). The pH was adjusted to 7.0 ± 0.1 with NaOH or HCl. The medium was autoclaved at 121 °C for 15 min. The glucose solution was filter-sterilized through 0.22 μm filters (Millipore polyvinylidene difluoride (PVDF) membrane). The MFC cultivation medium for both microbial consortia systems consisted of glucose to energize the bacterial growth in the first 12 h operation. Then, 10 mg/L 2,4-DCP solution was added to the growth medium of both microbial consortia systems (henceforth referred to as 'phenolic feeding'). In contrast, the 'non-phenolic fed' MFC only received glucose feeding after the first 12 h and received no added phenolics. 50 mM phosphate buffer was used as the MFC catholyte in this study.

2.2. Microbial community analysis and characterization

2.2.1. DNA analysis of microbial community

The biofilms on the anode surface and the suspended cells in the anolyte of both phenolic-fed and non-phenolic-fed MFCs were sampled for microbial community analysis. The bacterial genomic DNA was extracted from 0.5 ml of cell suspension and 0.5 g of dried biofilm for each sample using FastDNA™ SPIN kits and the FastPrep® Instrument (MP Biomedicals, Santa Ana, CA). The bacterial V3-4 hypervariable region of the bacterial small ribosomal subunit coding gene (SSU) was amplified via polymerase chain reaction (PCR) performed on the DNA extracts. The products of all samples were then multiplexed as described in Vasileiadis et al. [16] and sequenced with the Illumina HiSeq 2500 platform for 300 cycles using the paired-end reads module by Fasteris SA (Geneva, Switzerland). The sequences were analysed with the LotuS v1.44 software suit [18]. Analysis steps included: de-multiplexing the sequencing reads to the samples of origin; quality control with the LotuS native simple de-multiplexer (smd) v1.26 using the default parameters with the exception of a minimum good quality sequence length of 170 bp; merging of read pairs with FLASH v1.2.8 [19]; clustering of reads into 97% identity operational taxonomic units (OTUs) with the USEARCH/UPARSE v8.0.1623 algorithm [20]; removal of chimeric OTUs with UCHIME v4.2 [21] and the ribosomal database project (RDP) gold database version; and representative sequence classification of OTUs using the RDP naïve Bayesian classifier v2.11 [22] and the SILVA v123 database [23]. The output matrices were analysed using the R v3.2.3 software [24] for: addressing sample relations via hierarchical clustering and the unweighted pair group method with arithmetic mean (UPGMA); assessing treatment-related differential abundances of OTUs with Fisher's exact tests using the Holm correction for multiple hypothesis testing and the Edge R v3.12.0 package [25].

As indicated by the Good's coverage, the final per-sample sequence numbers were sufficient for screening the vast majority (97.2–100%) of the environmental bacterial diversity in these samples [26].

2.2.2. Characterization of biofilm morphology

The morphology of the DMC and IMC biofilms formed on the anode surfaces in the MFC systems was characterised using a Philips XL30 scanning electron microscope (SEM). The carbon cloth-anode attached biofilms were aseptically removed from the MFC anodic chamber and washed with phosphate buffer saline solution. The biofilm samples were then dried at 80 °C for 24 h and the samples were coated with platinum prior to high resolution SEM imaging.