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Three-dimensional nickel foam and graphene electrode in microbial fuel cell application: Study of biofilm compatibility

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Significance Statement

Current methods of domestic and industrial wastewater treatment are energy intensive and unsustainable, and microbial fuel cells have the potential to create a paradigm shift in wastewater treatment by generating energy from wastewater itself. This study investigates the potential of nickel foam and graphene as a possible electrode material.

Category: Experimental research

Keywords: **Microbial fuel cell, graphene synthesis, three-dimensional electrode, biofilm analysis**

Abstract

Microbial fuel cells (MFCs) have the potential to transform both domestic and industrial wastewater treatment sectors, posing an alternative to existing methods of wastewater treatment. MFC technology generates energy from wastewater by exploiting the properties of electroactive microorganisms to generate a current while simultaneously decomposing organic matter. MFCs are not yet practical for wide-scale implementation due to high material costs and system inefficiencies. The objective of this project was to synthesize an improved anode material optimized for MFC systems from nickel foam (NF) and graphene. NF/graphene materials offer a low-cost alternative to traditional precious metal electrodes and electrical properties more robust than carbon papers and foams. The biocompatibility of the synthesized anode with bacterial communities was evaluated, and biofilm growth on NF and graphene electrodes was measured both for pure bacterial strains and for environmental samples. Although additional experiments are required to draw definitive conclusions, preliminary results demonstrate antibacterial activity in the NF and graphene materials, which could hinder MFC performance (i.e. current generation).

1. Introduction

Microbial fuels cells (MFCs), a type of bioelectrochemical system (BES), have the ability to revolutionize traditional methods of wastewater treatment by generating energy through the use of electroactive bacterial biofilms. In contrast, current methods of wastewater treatment are energy intensive, depleting a limited fuel supply and releasing environmental pollutants. Due to escalating water management concerns, water treatment was identified by the National Academy of Engineering as an Engineering Grand Challenge [1]. Despite prior work, MFCs still suffer from a wide variety of inefficiencies that prevent their immediate use in wastewater remediation at the industrial scale. Inefficiencies include both the slow rate of anaerobic respiration at the anode and formation of the biofilm itself [2]. Improvements to the anode material could mitigate these issues, increasing feasibility of wide-scale implementation of MFC technology. The objective of this project was to develop more effective anode materials for implementation in an MFC system and to analyze the compatibility of these anode materials with bacterial communities.

Carbon materials, including three-dimensional carbon foams, have routinely been explored as a potential anode material for MFC systems, and Chen et al. found that reticulated carbon foam derived from a sponge-like natural product performed relatively well in an MFC application, with a current density of over 4.0 mA cm^{-2} [3]. Similarly, Zhao et al. identified a layer by layer synthesis method of three-dimensional nickel foam (NF) electrodes coated with graphene layers for use in oxygen evolution reactions, and electrical properties of the synthesized electrodes were comparable to that of state-of-the-art materials including Ir/C and Ru/C electrodes, demonstrating a notable current density of 10 mA cm^{-2} [4]. Although the Zhao procedure offers an economical three-dimensional anode material with superior electrical

properties, the potential for use in an MFC setting has not yet been explored and could offer interesting results.

The success of MFC technology fundamentally depends on the ability of electroactive bacteria, typically organized in a biofilm formation on the surface of the anode, to oxidize organic materials. Current density has been shown to have a positive correlation to biomass [2]. The present study completes a preliminary biocompatibility analysis of NF/graphene electrode material prepared using a modification of the Zhao procedure with analytical methods including planktonic cell counts and confocal microscopy [4]. This analysis is relevant due to the known toxicity of nickel and due to the compulsory biocompatibility of materials in MFC design and preparation [5]. It was hypothesized that antibacterial activity would be observed.

2. Materials and methods

Modifications were made to the Zhao procedure to optimize the synthesized material for use in an MFC system [4]. NF and graphene electrode samples of 1 cm by 1 cm were created by first submerging NF in acetone for 20 minutes, rinsing with deionized water, submerging in 0.10 M HCl for 20 minutes, and rinsing again with deionized water. After cleaning the NF base, graphite oxide (GO) layers were cyclically developed on the surface of the NF. Electrodes were submerged in a 6.25 mg/mL solution of poly(ethyleneimine) (PEI) at a pH of 10 for 20 minutes. The PEI solution was held at pH 10 rather than pH 7 due to the weak polyelectrolyte nature of PEI [4]. Following a rinse with deionized water, electrodes were then submerged in a graphite oxide (GO) suspension (GO-325) of 1.5 mg/mL at a pH>10 for 20 minutes. GO was prepared using a modified version of Hummer's method [6]. Electrodes were removed from the solution and air dried. Steps were repeated until a Carl Zeiss 0.1-30 kV scanning electron microscope (SEM) revealed a uniform graphene layer, between 5 and 7 times depending on the trial. The GO coating was reduced to graphene with functionalized nickel nanoparticles using a mix of 20 mL of "$Ni(NO_3)_2 \cdot 6H_2O$" 10 mM (58.2 mg/20 mL agua milli-Q) and 4 mL of L-ascorbic acid (LAA) 120 mg/mL. Electrodes were left in the solution for 4 hours at 80°C under gentle magnetic stirring. LAA was selected as the reducing agent rather than hydrazine as used by Zhao et al. because it exhibits a significantly lower inherent toxicity, enabling the implementation of the synthesized material in biological systems [4,7]. All chemicals used in this procedure were of high purity reagents and were purchased from Sigma-Aldrich (Chile).

Following UV radiation for the purpose of sterilization, 2 NF and graphene electrodes were introduced in each of 6 cultures: Tryptic Soy Broth (TSB) only, Streptomyces sp., Pectobacterium sp., wastewater, sludge, and solid waste. A TSB control without NF/graphene electrodes was included as well. All environmental samples (wastewater, sludge, and solid waste) were collected from ESSBIO wastewater treatment plant facility in Concepción, Chile. Planktonic cell counts were collected at day 2 and at day 11 using an Olympus BX51 Epifluorescence Microscope at a magnification of 1000x, and biofilm images were collected at day 11 using a LSM780 NLO Zeiss Spectral Confocal Microscope at magnifications of 25x and 40x. Confocal microscopy images were analyzed by the Centro de Microscopía Avanzada at the Universidad de Concepción. The percent volume of live and dead cells relative to the total electrode volume were determined using manually set controls and were held constant across samples, except for Pectobacterium sp., which was analyzed using a different magnification. Additionally, planktonic cell counts were collected at day 4 for both plastic and carbon cloth supports, used as controls for the NF/graphene materials. The plastic supports are known to encourage biofilm growth, and carbon is relevant given previous work in the field. All samples were stained using a LIVE/DEAD® BacLight™ Bacterial Viability Kit (N° L13152) purchased from ThermoFisher (Chile).

3. Results

Images collected using SEM technology are provided for the NF/graphene material prior to biofilm growth in Figure 1, revealing a uniform graphene coating on the surface of the NF and validating the coating and reduction methods used in the material synthesis.

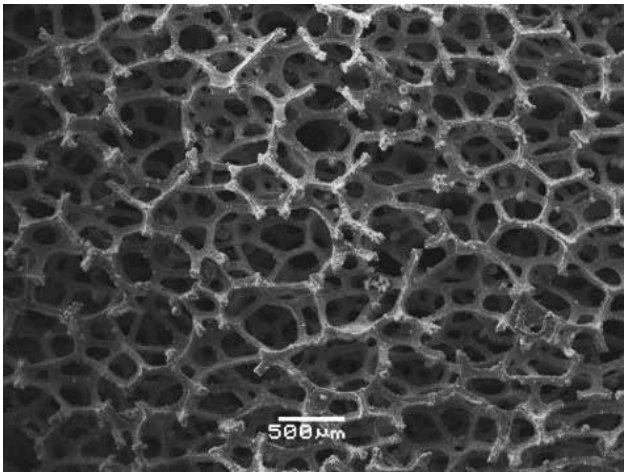

Figure 1: SEM imaging for GO reduced to graphene on NF. Uniform graphene coating after 5–7 cycles of PEI and graphite oxide, with LAA as the reducing agent.

Cell count data showed bacterial growth was absent from the TSB controls. Filaments present in the *Streptomyces sp.* culture prevented data collection for all types of biofilm supports and these data points are not included. Planktonic cell concentrations for day 2, day 4, and day 11 in are presented in Table 1 and Table 2, respectively. Reported planktonic cell concentrations were determined using the average of 4 manually collected counts, and 95% confidence intervals are included for each data point. Differences between days 2 and 11 are not statistically significant for Pectobacterium sp., while the cell counts between days 2 and 11 significantly increase for wastewater and sludge and significantly decrease for solid waste. However, both the carbon cloth and the plastic supports show consistently higher planktonic cell counts than the NF/graphene, reaching up to $7.94 \times 10^9 \pm 2.04 \times 10^9$ cell/mL.

	NF/Graphene (cell/mL)	
Culture	Day 2	Day 11
Pectobacterium sp.	$3.51\times10^{9} \pm 5.1\times10^{8}$	$3.45\times10^{9} \pm 5.3\times10^{8}$
Wastewater	$1.90\times10^{9} \pm 1.8\times10^{8}$	$3.42\times10^{9} \pm 4.6\times10^{8}$
Sludge	$2.27\times10^{9} \pm 2.5\times10^{8}$	$3.00\times10^{9} \pm 6.8\times10^{8}$
Solid waste	$3.46\times10^{9} \pm 2.3\times10^{8}$	$1.36\times10^{9} \pm 3.2\times10^{8}$

Table 1: Planktonic cell concentrations for live growth on NF/graphene

	Plastic (cell/mL)	Carbon Cloth (cell/mL)
Culture	Day 4	
Wastewater	$5.28\times10^{9} \pm 1.25\times10^{9}$	$5.20\times10^{9} \pm 1.11\times10^{9}$
Sludge	$5.72\times10^{9} \pm 1.79\times10^{9}$	$5.17\times10^{9} \pm 4.4\times10^{8}$
Solid waste	$7.94\times10^{9} \pm 2.04\times10^{9}$	$6.50\times10^{9} \pm 1.74\times10^{9}$

Table 2: Planktonic cell concentrations for live growth on support controls

Confocal microscopy images are included in Figure 2 for live cells at day 11 in the NF/graphene electrodes. Quantitative results for percent volume of live and dead cells are shown in Table 3. Overall, visual analysis revealed a higher concentration of live cells for the environmental samples than for *Pectobacterium sp.*

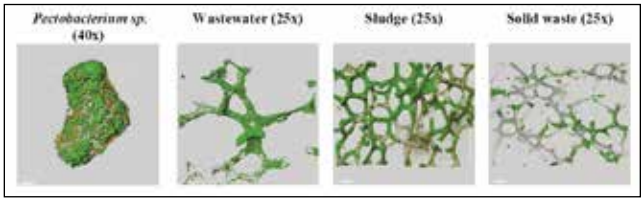

Figure 2: Confocal microscope images of adhered biofilm. Environmental samples provided more prominent biofilm than *Pectobacterium sp.* at day 11 of biofilm growth on NF/graphene.

Culture	Live (% volume)	Dead (% volume)
Pectobacterium sp.	10.58	11.08
Wastewater	28.49	7.48
Sludge	31.62	13.70
Solid waste	15.70	5.56

Table 3: Calculated biofilm growth on NF/graphene electrodes by percent total volume

4. Discussion

NF/graphene planktonic cell counts ranged from $1.36\times10^{9}\pm3.2\times10^{8}$ cell/mL to $3.51\times10^{9}\pm5.1\times10^{8}$ cell/mL, and both the plastic and the carbon cloth supports appeared to be more compatible with biofilm growth, reaching up to $7.94\times10^{9}\pm2.04\times10^{9}$ cell/mL and $6.50\times10^{9} \pm 1.74\times10^{9}$ cell/mL, respectively. Strictly within the NF/graphene materials, the planktonic cell counts across cultures were inconclusive. After 2 weeks of growth, confocal microscopy revealed biofilms colonizing from 10.58% to 31.62% of the total sample volume. These results, across all samples, were less prevalent than expected, supporting the likelihood of antibacterial activity in the NF/graphene electrodes. Similar work by Blanchet et al. reported biofilm growth of up to 39.3±1.1% on carbon cloth using activated sludge fed with food wastes, at nearly 10% greater than the 31.62% obtained by this work [8].

The environmental samples showed increased biofilm formation relative to the pure strain *Pectobacterium sp.*, likely due to interspecies cooperation and greater adaptability within a multispecies biofilm [9]. This is observed in the confocal microscopy images in Figure 2, as the density of living cells (green) appears lower for *Pectobacterium sp.* than for the environmental samples (wastewater, sludge, and solid waste). The observed toxicity of the NF/graphene electrodes may be due to the NF or graphene, as nickel is known for its toxic effects and graphene has been shown to cause membrane and oxidative stress in bacterial cells [5,10]. It is recommended that the antibacterial effects of the synthesized materials be further explored with more extensive controls and larger sample sizes, with a focus on mixed culture inoculants from environmental samples. Future work will focus on more extensive data collection, and controls will be used to isolate the particular source of antibacterial activity. However, it is possible that the increased electrical performance of NF/graphene electrode will allow for a small amount of remaining antibacterial activity in the materials. Electrical activity could compensate for a slight decrease in biofilm growth, and a valuable MFC system will demonstrate improved current generation.

5. Conclusions

This work focused on a preliminary analysis of the antibacterial effects of NF and graphene-based anode materials synthesized using a modification of the procedure originally presented by Zhao et al. [4]. Pure strains and mixed cultures were included in the analysis, with biofilms grown using *Pectobacterium sp., Streptomyces sp.*, and 3 types of environmental samples (wastewater, sludge, and solid waste) collected from the ESSBIO wastewater treatment plant facility in Concepción, Chile. Initial results support the presence of inherent antibacterial activity in the NF/graphene materials, although further work is required to verify these conclusions. Particularly, more thorough controls need to be tested to isolate and to subsequently reduce the source of the toxicity. However, the enhanced electrical properties of NF/graphene electrodes could compensate for a minimal degree of antibacterial activity, and NF/graphene materials warrant future investigation. Developments in this technology could allow for the eventual implementation of MFCs in both municipal and industrial sectors, significantly lowering the energy cost of current wastewater treatment methods.

6. Acknowledgements

This work was funded by the NSF REU Project #1757529 and the National Commission for Scientific and Technological Research FONDEF IDEA No. ID18I10337, Concepción, Chile. Claire Chouinard would like to acknowledge the University of Maine FBRI REU program, as well its directors, Dr. Douglas Gardner and Dr. David Neivandt. She would also like to thank Dr. David Sanchez.

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