Bioresource Technology 256 (2018) 195–200

Contents lists available at ScienceDirect

Bioresource Technology

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

Rewiring the microbe-electrode interfaces with biologically reduced graphene oxide for improved bioelectrocatalysis

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ARTICLE INFO

Keywords:
Bioelectrochemical systems
Reduced graphene oxide
Biocompatibility
Bioelectrocatalysis
Current density
Electron transfer rates

ABSTRACT

The aim of this work was to study biologically reduced graphene oxide (RGO) for engineering the surface architecture of the bioelectrodes to improve the performance of Bioelectrochemical System (BES). *Gluconobacter roseus* mediates the reduction of graphene oxide (GO). The RGO modified bioelectrodes produced a current density of 1 mA/cm^2 and 0.69 mA/cm^2 with ethanol and glucose as substrates, respectively. The current density of RGO modified electrodes was nearly 10-times higher than the controls. This study, for the first time, reports a new strategy to improve the yield as well as efficiency of the BES by wrapping and wiring the electroactive microorganisms to the electrode surfaces using RGO. This innovative wrapping approach will decrease the loss of electrons in the microbe-electrolyte interfaces as well as increase the electron transfer rates at the microorganism-electrode interfaces.

1. Introduction

Bioelectrochemical systems (BES) are promising technologies for diverse applications with capabilities to operate in a broad range of environments including space and deep biospheres (Venkata Mohan et al., 2014). Apart from bioelectricity generation, BES finds applications in effluent treatment (Yeruva et al., 2015; Watson et al., 2015; Nancharaiah et al., 2016), desalination (Nikhil et al., 2016), metals removal and recovery (Nancharaiah et al., 2015) and production of value-added products (Roy et al., 2016). The use of the electroactive microorganisms or enzymes as electrocatalysts makes the bioelectrochemical processes economical and eco-friendly when compared with the chemical, electrochemical, and microbial processes. The use of electroactive microorganisms in BES helps in catalysing a broad range of substrates and accelerate the rates of electrocatalysis. BES suffers from low rates of bioelectrocatalysis when compared to other non-enzymatic electrocatalytic processes. Several approaches including the identification of new electroactive microorganism (Bhuvaneswari et al., 2013), new substrates (Thygesen et al., 2009; Selvaraj et al., 2016), surface display technology (Fishilevich et al., 2009), metabolic engineering approach (Gustavsson and Lee, 2016), improving the fuel cell configuration (Cui et al., 2016; An et al., 2016; Navanietha Krishnaraj et al., 2013; Navanietha Krishnaraj et al., 2015a), proton-exchange membrane (PEM) (Daries Bella et al., 2016) have been reported to improve the performance of BES. However, most of these strategies could not drastically increase the rates of bioelectrocatalysis.

Electron transfer resistance across electroactive microorganism-electrode interfaces is one of the major shortcomings impeding the rates of bioelectrocatalysis. Choice of an electrode material that aids the anchoring of bacteria, facilitating direct electron transfer, will greatly lessen the interfacial electron transfer resistance at the microorganism-electrode interfaces (Choi and Sang, 2016). Microbes have been shown to respire onto the electrodes with the aid of their inbuilt conductive membrane proteins such as cytochromes and conductive pili nanowires (Lovley, 2011). Harnessing the maximum number of electrons between the microbe and the electrodes has been a challenge. Conductive membrane proteins that are involved in receiving/transferring the electrons from/to electrodes are orientated on the surface of the microbial cells. A major fraction of the cell surface containing these proteins are exposed to the electrolyte, and are not attached to the electrode to mediate direct electron transfer. Technologies that could aid in trapping most of the electrons from the surface of entire microbial cells would significantly increase the yield of BES. Although electrode functionalizing strategies helped to increase the adherence of bacteria, increasing electron transfer rates, they did not attempt to trap the electrons produced by the microorganisms completely (Navanietha

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https://doi.org/10.1016/j.biortech.2018.02.001
Received 29 November 2017; Received in revised form 31 January 2018; Accepted 1 February 2018

Krishnaraj et al., 2013).

Reports have been made on the use of materials with high conductivity and high specific surface area such as graphite, carbon foam, carbon felt, and carbon paper to increase the yield of electrocatalysis (Wei et al., 2011). These materials increase the yield and electron transfer rates, but loss of electrons on the microbe-electrolyte interface remains. In addition, certain carbon electrode materials fail to support the adherence of bacteria to its surface (Cornejo et al., 2015). Functionalizing the electrodes using biopolymers, nanomaterials, and conducting polymers have been reported to increase the biocompatibility of the electrodes thus improving the performance of BES (Karthikeyan et al., 2016). But but the biopolymer modified electrodes do not improve the electron transfer characteristics of the electrode. On the other hand, the use of nanomaterials and conducting polymers for modifying electrodes, pose toxicity to the microbial cells leading to a decrease in rates of microbial catalysis (Kang et al., 2008). These methods aim to improve the adhesion of microorganisms, or rates of electron transfer leading to improved electrocatalysis, but they fail to focus on trapping the maximum number of electrons that are produced by the microorganisms, leading to poor efficiency of the system.

Herein, the use of graphene for wrapping the microorganisms, which can aid in efficiently trapping the electrons from the entire surface of the microorganisms is reported. The extraordinary features of graphene, including high electrical conductivity and large surface area, make it a promising material for wiring the microorganisms to the electrode surface (Zhu et al., 2010). Chemical methods that employ reducing agents such as hydrazine (N_2H_4), dimethylhydrazine ($C_2H_8N_2$), and sodium borohydride ($NaBH_4$) may not yield biocompatible RGO that is desired for wrapping the microbial cells (Chua and Pumera, 2014; Barbolina et al., 2016; Qiu et al., 2017). Graphene produced by these chemical routes is also prone to lose its unique properties such as high conductivity that is crucial for electrochemical applications (Stankovich et al., 2006b; Stankovich et al., 2007). In addition, chemical reducing agents like hydrazine and hydrazine hydrate are toxic and explosive, and may pose several deleterious effects to the environment. On the other hand, reports are documented on the reduction of GO using microorganisms such as *Shewanella* sp. (Salas et al., 2010; Wang et al., 2011) and *E. coli* (Akhavan and Ghaderi, 2012; Gurunathan et al., 2013).

Biologically reduced RGO was shown to enhance the extracellular electron transfer in microbial fuel cells. Simultaneous reduction of GO and current generation in the anodic compartment of microbial fuel cell is also reported (Yuan et al., 2012). This study reports, for the first-time wiring the RGO wrapped microorganisms with electrodes, which reduces the loss of electrons in the microbe-electrolyte interfaces as well as increases the electron transfer rates at the microorganism-electrode interfaces, leading to improved electrocatalysis.

2. Materials and methods

2.1. Synthesis of graphene oxide

Graphite powder of size less than 20 μm, purchased from Sigma Aldrich, was used for the synthesis of GO. Graphene oxide was synthesized using the modified Hummers method (Hummers and Offeman, 1958). Briefly, 4 g of graphite and 2 g of sodium nitrate were added to a 250-mL flask. 100 mL of concentrated H_2SO_4 was slowly added. Stirring was continued for 30 min 0.6 g of $KMnO_4$ was added to the mixture with continuous stirring for another 30 min 14 g of $KMnO_4$ was added to the mixture. Stirring was continued for few hours till the temperature of the mixture comes down to room temperature. 180 mL of water was slowly dripped into the paste and the diluted suspension was once again stirred for another 15 min. Finally, 7 mL of H_2O_2 and 55 mL of water was added. The resulting bright yellow solution was filtered and the yellow brown filter cake was collected. The cake was washed repeatedly with 3% aqueous HCl. The synthesized GO was dried in a desiccator and is used for the synthesis of graphene.

Carbon felt with a geometrical area of 0.4 cm^2 was used for the experiment. The electrical connection was made with a brass rod. The fabricated carbon felt electrodes were cleaned, and were used as bare electrodes. Carbon felt electrode of same size was modified with RGO.

2.2. Reduction of GO

Reduction of GO was mediated by pure cultures of *G. roseus* (National Chemical Laboratory, NCIM #2524). The culture was inoculated into a growth medium containing tryptone (1 g), yeast extract (1 g), glucose (1 g), and $CaCO_3$ (1 g) in 100 mL of distilled water and incubated for 24 h at 37 °C. After 24 h, the broth containing the microbial cells was centrifuged, and the cells are washed with phosphate buffer (0.1 M, pH 7) to remove the debris and medium constituents. About 0.1g (wet weight) cells were dispersed in phosphate buffer containing sorbitol (5g/100 mL) as the electron donor (substrate) and 0.5g of GO as the electron acceptor. The conical flask was kept in the incubator at 37 °C for 24 h for the reduction of GO. The Scanning Electron Microscopy (SEM) images of the bacteria wrapped with RGO during the reduction process were observed. *G. roseus* reduced graphene oxide was collected and purified by washing repeatedly with deionized water to remove the cell debris. The purified samples were collected and washed in the following sequence: 18 MΩ-cm water, 80% ethanol, 18 MΩ-cm water, 1 N HCl, and 18 MΩ-cm water (Millipore) (Salas et al., 2010).

2.3. Characterization of RGO

The synthesized GO and RGO were characterized by UV spectroscopy, Fourier Transform Infrared (FT-IR) spectroscopy, Laser Raman spectroscopy, and X-ray powder diffraction. FT-IR spectra of the GO and RGO were recorded in a spectral range of 400–4000 cm^{-1} using FT-IR Spectrometer (Bruker Optik GmbH, Germany, TENSOR 27). The samples for FT-IR were prepared by grinding the dry powdered sample with KBr. The powdered samples were smeared on a clean SiO_2/Si substrate that was used for the Raman measurement. X-ray powder diffraction spectra of both the samples were recorded to observe the diffraction features (Bruker). The synthesized GO and RGO were characterized by Transmission electron microscopy (FEI, Tecnai 20 G2). The samples for TEM characterization were prepared by placing the aqueous suspension (~0.02 mg/mL) of GO or RGO samples on the Cu grids and allowed to dry. About 0.5 mg RGO (in 1 mL of phosphate buffer (0.1 M, pH 7)) was drop casted on the carbon felt electrode. Adhering bacteria onto the RGO electrode had the tendency to wrap the RGO on its surface. This leads to natural wiring of the microbial conductive proteins on the surface to the electrodes. The bare and modified electrodes were analysed using SEM.

2.4. Formation of biofilm

The biofilm of a mixed culture containing *Acetobacter aceti* and *G. roseus* was formed on both bare and modified electrodes in phosphate buffer solution, containing glucose (0.2 g/30 mL of buffer) and the mixed cultures were incubated under anaerobic conditions. Biofilm was allowed to form until the electrode reached a stable negative potential. The electrodes with biofilms were used for studying the efficacy of the functionalized electrodes on microbial electrocatalysis of ethanol. Similarly, natural biofilm formed from a food waste sample was formed on bare and modified electrodes with the food waste sample. The electrodes with natural biofilm were also used for analysis of microbial electrocatalysis of glucose.

2.5. Electrochemical studies

The bare and modified electrodes containing the biofilms were