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Screening anodic inoculums for microbial fuel cells by quantifying bioelectrogenic activity using tungsten trioxide quantum rods

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ABSTRACT

The aim of this research was to develop a procedure for rapid detection of electrogenic activity of the inoculums using electrochromic tungsten trioxide (WO_3) quantum rods, which display blue colouration under an electric field. Optimum dosage of WO_3 and sludge concentration to be used for absorbance measurement were determined to estimate electrogenic activity based on blue colouration. Performance of seven MFCs was evaluated using different volumetric ratios of mixed anaerobic sewage sludge and *Shewanella putrefaciens* as inoculums. Absorbance of inoculums used in MFCs was measured after addition of optimum dosage of WO_3 and a correlation is established between this absorbance and respective current densities/CE, obtained in MFCs during steady state operation. This correlation of current densities/CE with absorbance of inoculum can be used to select proper inoculum having highest electrogenic activity, and to predict possible current generation of MFCs, even before start-up, based on the electrogenic activity of inoculum used.

1. Introduction

The development of microbial fuel cell (MFC) concept was materialized to serve principally three purposes; first, to offer effective anaerobic treatment of different organic wastes; second, for energy or by-products recovery from waste; and the last but not the least is to develop environment specific green technology (Dresselhaus and Thomas (2001); Logan, 2008; Lovley, 2006). Bacteria, more precisely gram negative electrogenic bacteria, are the soul of this biological system, which participate as the biocatalyst in an MFC and forms an anodic biofilm to treat available organic waste as a part of their own metabolism (Logan and Regan, 2006). Bacterial metabolism refers to all the biochemical reactions that occur in a bacterial cell to obtain energy majorly from carbon source (Xu et al., 2016). Electrogenic bacteria present in anodic biofilm of MFC respires through anodic respiration process in absence of oxygen or other alternate electron acceptor using physolmere electron transport chain process and transfer electrons from oxidized compounds to electrode (Mercuri et al., 2016; Rittmann et al., 2008). This extracellular electron transfer (EET) process between bacteria to electrode has received significant importance in various biochemical applications including in MFC to generate electricity (Kumar et al., 2016; Reguera et al., 2005).

Identification of this extracellular electroactive bacterial inoculum source is critical to overcome from the constraint of performance uncertainty of the MFC to generate power for practical applications. Various time consuming and expensive techniques are available for the detection of electrogenic bacteria such as DNA isolation of bacteria followed by 16S rRNA gene amplification, sequencing and bioinformatics and phylogenetic analyses of bacterial strain, printed paper- and transparency-based analytic devices, immunological, mass spectrometry, Raman scattering spectroscopy, and the most probable number (MPN)-PCR method (Adkins et al., 2017; Liu et al., 2011; Taratus et al., 2000; Yang et al., 2016; Yu et al., 2012; Zhang et al., 2013). However, these or similar other methods require expensive chemical, instrument, as well as long time span to complete those multi-step experiments. Opposite to above mentioned methods that can be adopted for electrogenic bacteria determination, semiconductor quantum rods such as tungsten trioxide (WO_3) are inexpensive, easy to synthesize, biocompatible, possess photo and electro-catalytic properties (Wang et al., 2015) and change its coloration to blue under an electrogenic environment; hence, these sensitive properties can be explored to determine the presence of electrogenic bacterial community.

Quantum dots are a novel class of inorganic semiconducting nanomaterials from the II-IV, IV-VI, or III-V group of Periodic table and have size controlled properties such as variation in pH can achieve desired morphology to target specific configuration, which captivated the considerable attention of researchers for existing and emerging technologies for bioanalytical and biomedical applications (Park et al.,

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2017). These semiconductor nano-materials have the ability to achieve rod shaped morphology, which is referred hereafter as quantum rods. These quantum rods nanoparticles are preferably in the size of below 2 nm and can have maximum size of 6 nm; hence, due to their similarities in dimensions with biological molecules, quantum rods are being used in various advanced medical imaging or biological labelling techniques (Jin et al., 2011). A 3-D Quantum confinement also provides unique electronic and optical properties to quantum rods, which makes them more advantageous over current biological detection methods such as lanthanide chelates, organic dyes or other fluorophores such as fluorescent proteins (Jin et al., 2011).

Among the several quantum rods nano-materials, tungsten trioxide, a n-type semiconductor has gained attention because of its outstanding photo-electro-catalytic, physicochemical and electrochromic properties (Jiao et al., 2010). Tungsten trioxide quantum rods are ultra-fine electroluminescent nanomaterial, which changes its colour to blue under electrical surrounding due to radiative recombination of an electron to it surface (Salmaoui et al., 2013). This electroluminescent property of WO_3 quantum rods was applied to determine electrogenesis in bacterial inoculum in a short span of time before their inoculation in MFC to ensure power production (Sharma et al., 2016). Pre-determination of electrogenic activity of inoculum using WO_3 quantum rods can reduce start-up time of MFCs, and proper selection of the inoculum will ensure electrogenic biofilm formation to achieve substantial power production. However, apart from determination of bioelectrogenesis, quantification of same i.e. quantitative assessment of electrogenic activity of the bacteria present in the inoculum is also an important factor to get a perception of inoculum performance to emphasize the utility of the application of WO_3 quantum rods for selection of inoculum for MFCs.

Therefore, the present study was aimed at adding value to previous study of bioelectrogenesis determination (Sharma et al., 2016) by quantifying electrogenic activity of the inoculum using WO_3 quantum rods. The research work is divided into three parts; first, an optimization of WO_3 quantum rods dosage and sludge concentration to be used based on blue colouration development, for which pure culture of *Shewanella putrefaciens* (renowned electrogenic bacteria) was used. In second stage, power performance of seven identical dual chambered MFCs having different percentages of mixed anaerobic sewage sludge and *S. putrefaciens* bacteria in inoculum was evaluated. Absorbance of inoculums used in these MFCs were recorded after adding optimized WO_3 quantum rods dosage, and lastly a correlation was established between absorbance and respective current densities (mA/m^2)/ coulombic efficiencies (CE%) obtained in MFCs. This strategy facilitates a rapid electrogenic activity estimation of inoculum by adding electrochromic WO_3 quantum rods and a prediction of current density and CE of MFC before actual operation.

2. Material and methods

2.1. Tungsten trioxide quantum rods synthesis and characterization

The electrochromic nanowires of WO_3 quantum rods were hydrothermally (calcination) synthesized using sodium tungstate dihydrate ($Na_2WO_4{\cdot}2H_2O$), a water soluble salt precursor (Sharma et al., 2016). Ultrapure Milipore MQ (Milli-Q®, Merck, Germany) deionized water was used for the preparation of all solutions to synthesize semiconductor electrochromic WO_3 quantum rods. In 50 ml of deionized water, 1.65 g of sodium tungstate (Sigma-Aldrich) and 0.58 g of sodium chloride (Sigma-Aldrich) were dissolved using magnetic stirrer (100 rpm). Drop by drop addition of 3 M HCl (Merck 35%) was done in above solution under continuous stirring till pH reached to 2.5–3. This solution was transferred to a Teflon autoclave, sealed and put in a preheated oven at 180 °C for 16 h, maintaining the same temperature for hydrothermal synthesis. After proper cooling, white precipitates of WO_3 quantum rods were collected. Cooling of solution inside the oven is important for proper precipitation of the WO_3 quantum rods under gradually decreasing temperature. This process of fabrication is also known as the bottom-up method in which self-assembly of chemical molecules form nanostructures due to solution-liquid–solid (SLS) process of synthesis. Collected precipitates were washed several times using centrifugation at 3000 rpm using deionized water. After adequate washing, the precipitate was transferred to a 50 ml glass beaker and dried at 60 °C in an oven for 24 h.

The crystal structure of synthesized quantum rods was characterized using selected area electron diffraction (SAED) and X-ray diffraction (XRD). Chemical characteristics and material verification were ascertained using energy dispersive X-ray spectroscopy (EDX) and chemical bonding using Fourier transform infrared spectroscopy (FTIR). The surface morphology of WO_3 quantum rods was observed under scanning electron microscopy (SEM). Further size distribution and morphology of nanomaterial surface were imaged using high-resolution transmission electron microscopy (HRTEM). The samples preparation and measurements were performed ex-situ at room temperature. The XRD of WO_3 nanomaterial was performed using Phillips, Netherland X'Pert PRO MRD (PW3050) diffractometer having Cu Kα radiation ($\lambda = 0.1541$ nm) from the 2θ range of 5°–70° and with a 0.03°/min scan rate. For the infrared spectra (IR), the FTIR analysis was done under atmospheric conditions using a NEXUS-870 FTIR spectrometer (Thermo Nicolet Corporation, USA), following potassium bromide (KBr) pellet technique in the spectral range of 400–4000 cm^{-1} with a resolution of 0.5 cm^{-1}. SEM analysis was done after gold sputtering using HITACHI E-101 ion sputter (Singapore) maintained at 0.1–0.01 Torr vacuum for a uniform gold coating of 300–350 Å and followed by imaging using Zeiss EVO 40 scanning electron microscope (Zeiss, EVO-40 SEM, Germany) having working distance of 6 mm with incident electron beam energy of 10 keV. Simultaneously while SEM imaging, EDX analysis of WO_3 quantum rods was performed using energy-dispersive X-ray spectroscopy (Oxford, UK) attached with SEM. The HRTEM and SAED images of nano quantum rods of WO_3 were revealed using JEOL-JEM-200 (MA, USA) at 200 kV operating voltage.

2.2. Inoculum and tungsten trioxide dosing

S. putrefaciens, an electrogenic bacterial culture (MTCC-8104) was used to determine the electrochromic-optical effect of WO_3 quantum rods. The bacterial culture of *S. putrefaciens* preserved in 10% glycol for 48 h, was grown on petri-plates containing autoclaved (121 °C for 15 min) media having yeast extract (2.0 g), peptone (5.0 g), beef extract (1.0 g), NaCl (5.0 g), agar (15.0 g), and distilled water (1.0 L). The matured colonies were transferred to 100 ml nutrient broth (Hi-Media) in 250 ml conical flask and put in a shaker-incubator at 37 °C at 150 rpm. The CFUs of *S. putrefaciens* broth were counted by plating method on nutrient agar petri plates. The serial dilutions of this exponentially grown bacterial broth from 10 to 100% with 10% interval were done using HEPES buffer, to make final volume of 10 ml, consisting of 5.85 g NaCl, 11.91 g HEPES (Sigma-Aldrich), 2.02 g sodium acetate, 0.3 g NaOH, 0.67 g $NaH_2PO_4{\cdot}2H_2O$, 1.498 g NH_4Cl, 0.097 KCl, and 0.4 ml trace metals per liter. Tungsten trioxide quantum rods solution in HEPES buffer was prepared using 2.0 g synthesized quantum rods in 20 ml of HEPES buffer and it was autoclaved before adding in tubes containing bacterial broth to determine bio-electrogenesis.

2.3. Spectrophotometric observations

A T80 high-performance double beam UV-Vis spectrophotometer (PG instruments, UK) having wave length range of 190–1100 nm was used to take the photometric absorbance measurement of the electrogenic bacterial population after addition of WO_3 quantum rods. Slow scanning was performed for each time to measure the suitable wavelength of blue colouration of samples after adding varied dosage of WO_3 quantum rods. Absorbance of other constituents such as HEPES buffer,