Chemosphere 174 (2017) 58–65

Contents lists available at ScienceDirect

Chemosphere

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

Formation of novel hydrogel bio-anode by immobilization of biocatalyst in alginate/polyaniline/titanium-dioxide/graphite composites and its electrical performance

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HIGHLIGHTS

- Conductive alginate/polyaniline/TiO_2/graphite composites were successfully synthesized.
- Optimised concentrations of aniline and graphite were determined.
- Novel bio-anode was fabricated by immobilisation of bacteria cells using conductive composites.
- Improvement of power density and stability of the microbial fuel cell was observed.

ARTICLE INFO

Article history:
Received 10 September 2016
Received in revised form
17 January 2017
Accepted 19 January 2017
Available online 23 January 2017

Handling Editor: J. de Boer

Keywords:
Microbial fuel cell
Shewanella algae
Alginate/polyaniline/titanium-dioxide/graphite
Immobilized cells
Conductive hydrogel
Bio-anode

ABSTRACT

A new bio-anode containing gel-entrapped bacteria in alginate/polyaniline/TiO_2/graphite composites was constructed and electrically investigated. Alginate as dopant and template as well as entrapped gel was used for immobilization of microorganism cells. Increase of polyaniline concentration resulted an increase in the conductivity in gels. Addition of 0.01 and 0.02 g/mL polyaniline caused 6-fold and 10-fold higher conductivity, respectively. Furthermore, addition of 0.05 g/mL graphite powder caused 10-fold higher conductivity and 4-fold higher power density, respectively. The combination of polyaniline and graphite resulted 105-fold higher conductivity and 7-fold higher power-density output. Optimized concentrations of polyaniline and graphite powder were determined to be 0.02 g/mL and 0.05 g/mL, respectively. Modified hydrogel anode was successfully used in microbial fuel cell systems both in semi- and continuous operations modes. In semi-continuous mode, about 7.88 W/m^3 power density was obtained after 13 h of fermentation. The glucose consumption rate was calculated to be about 7 mg glucose/h/$1.2 \cdot 10^7$ CFU immobilized cells. Similar power density was observed in the continuous operation mode of the microbial fuel cell, and it was operated stably for more than 7 days. Our results are very promising for development of an improved microbial fuel cell with new type of bio-anode that have higher power density and can operate for long term.

1. Introduction

Microbial fuel cell (MFC) is considered to be an attractive and promising sustainable technology because of application of electrochemically active microorganisms that are able to convert directly chemical energy (in organic chemical bounds) to electrical energy (Debabov, 2008; Kim et al., 2007). Meanwhile, this technology may have a big potential for extracting electric energy (Rahimnejad et al., 2011; Koók et al., 2016) from organic wastes or other compounds, or in bioremediation etc. (Logan et al., 2006; Pant et al., 2010) and production of some valuable substances such us biohydrogen (Zhen et al., 2016a) or biomethane gas (Zhen et al., 2016b), however its application is still limited due to the low power density and poor long-term stability (Liu et al., 2014).

Abbreviations: MFC, Microbial fuel cell; PANI, Polyaniline; PAOA, poly(aniline-co-o-aminophenol); PEM, proton exchange membrane; CSTR, continuous flow stirred-tank reactor; CNP, carbon nanopipes; GNS, graphite nanosheets; LB, Luria-Bertrani; DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH; MO, metal-oxide.

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http://dx.doi.org/10.1016/j.chemosphere.2017.01.095
0045-6535/

No doubt that one of the main limiting factors is the anode, where the biocatalysts (bacteria) generate exo-electrons and transfer them to the anode. The efficiency and capacity of the anode strongly depend on two factors: capacity of the applied biocatalyst (Hu, 2008; Oliveira et al., 2013) and electron transfer process (Osman et al., 2010; Li et al., 2011a). The properties of the used microorganism are determinative, since the electron transfer efficiency is recognized to be essential in increasing current and thus improving the MFC performance (Busalmen et al., 2008). Some mechanisms of electron transferring method in anodic chamber are reported: direct electron transfer via cell membrane proteins (Bond and Lovley, 2003), via conductive nanowires (Gorby et al., 2006), or indirect electron transfer via metabolites as electron shuttle molecules and artificial mediators (Du et al., 2007; Park and Zeikus, 2000). Various strategies have been developed to improve electron transfer in MFC such as application of different chemical catalysts, bioactive redox compounds, modification of electrodes etc. (Liu et al., 2013; Qiao et al., 2007; Rosenbaum et al., 2007; Zhao et al., 2010). Generally, *Shewanella* (Pinchuk et al., 2010; Li et al., 2011b) and *Geobacter* (Logan et al., 2006; Trinh et al., 2009) species (commonly used microorganisms in MFC systems) attach directly to electrode forming electro-conductive biofilm on the anode surface, thus in these cases, the performance of MFC is strongly affected by the quality of the biofilm layer(s) (Lovley, 2008) and the surface of the anode (Chen et al., 2014), and in somehow the distance between microorganism cells and electrode.

In the last decade, due to the intensive development of nanotechnological materials the anode engineering of the MFC has turned onto a new stage. Several reports have been published related to this (Li et al., 2011a, 2012; Qiao et al., 2007; Sapurina and Shishov, 2012). Polyaniline (PANI) is well known as a highly conductive polymer that has mechanical flexibility and stability, resistance to decomposition, and it can be easily tailored to various levels and produced with relatively low cost (Sapurina and Shishov, 2012; Guimard et al., 2007; Su et al., 2007). Additionally, these properties can be enhanced by exploration of the interaction of PANI and other inorganic compounds such as metal oxides (MOs), nanocarbon tubes, graphite/graphene etc. (Lokesh et al., 2008; Wang and Ren, 2013; Hou et al., 2013). The PANI coated anodes are successfully used to shuttle electrons from the bacterial suspension to the anode (Lai et al., 2011; Logan, 2008; Schroder et al., 2003) to improve power density of the MFC. Due to difficulty to create chemical interactions between PANI and MOs, synthesis of PANI-MO composites still has many challenges. Practically, surfactants such as camphor, dodecyl benzene and naphthalene sulphuric acid have been used as both dopants and templates to enhance the formation of PANI-MO composites. These compounds are known to cause environmental problems, thus avoidance of the possible environmental hazards is much more welcome in biotechnological developments.

Alginate, a water-soluble and heteropolysaccharide polymer composed of β-D-mannuronic acid and α-L-guluronic acid, widely distributed in diverse seaweeds and bacteria. This polymer is commercially available as sodium alginate and has been widely used in medicine, food and pharmaceutical industries as thickening, emulsifying, film forming and gelling agents (Fraser and Bickerstaff, 1997). Furthermore, entrapment in insoluble calcium alginate gel is recognized as a rapid, non-toxic, inexpensive and versatile method for immobilization of microorganism cells (Fraser and Bickerstaff, 1997). From these points of view, application of alginate as template to synthetize PANI-MO composite as well as carrier to immobilize cells for formation of bio-anode of MFC is definitely attractive because of its compatibility with environmental and biological systems (Lokesh et al., 2008; Fraser and Bickerstaff, 1997).

Main goal of this study focused on fabrication and application of novel hydrogel bio-anode by immobilization of biocatalysts in alginate/PANI/TiO_2/graphite composites, as well as on the investigation of electrical properties of this bio-anode in different MFC systems.

2. Materials and methods

2.1. Microorganism

Shewanella algae DSMZ 9167 strain was purchased directly from German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). The capability of production of exo-electrons of this strain was first screened by photometric method reported earlier (Szöllősi et al., 2015).

2.2. Culturing conditions

The *Shewanella algae* DSMZ 9167 strain was inoculated and maintained in Bacto Marine Broth (DSMZ Medium 514 or DIFCO 2216), and was transferred into Luria-Bertrani (LB) broth, which contains 10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl, at 30 °C before use (Szöllősi et al., 2015).

2.3. Fabrication of composites coated hydrogels

The conductive hydrogels were prepared as previously described by Basavaraja et al. (2010) with some modifications. Briefly, 0.1 g Na-alginate (Cargill, Hungary) was first dissolved in 10 mL distilled water and then appropriate amounts of (0 mg, 1 mg or 2 mg) aniline (Merck, Hungary) were added to the mixture while mixing rigorously. The aniline-alginate network was formed by ultra-sonication (Clifton MU-8 sonicator, 40 kHz, 30 W) for 2 h (Fig. 1). The composites were synthesized by *in situ* doping polymerization in presence of Ti(IV)O_2 (anatase; VWR, Hungary) (in range of 0.1 g) and 0.1 g ammonium persulfate (Reanal, Hungary) using ultra-sonication (40 kHz, 30 W) for 2 h at −10 °C. In this process, the alginate acts simultaneously as dopant and template (Basavaraja et al., 2010). The gel network (cross linkers with Ca^{2+}) of composites (Fig. 1) was formed by addition of different amount of graphite powder (Pannoncolor, Hungary) (0 mg, 2 mg or 5 mg) which has an average size of 20–50 μm, and dropping in 10 mM calcium chloride (Reanal, Hungary) solution. In the case of preparation of bio-anode the suspension of the exo-electrogenic bacteria culture (*Shewanella algae*) at 10^7 CFU/mL was added with graphite powder. At the end of fabrication process, composites coated hydrogel particles (with 0.5 cm–0.8 cm in diameter) were formed (Fig. 1). The created composite gels were used directly as anode of the MFC system, so no other current collector was applied.

2.4. Batch, semi-continuous and continuous operation of the MFC

For the different operation modes the same type of MFC were applied. The gel-electrodes were investigated in dual-chamber MFCs (Fig. 2). The volume of each chambers was 12 mL (3 cm × 4 cm ×1 cm). The anodic chamber was filled approx. 50% with the gel-electrodes. The total area of surface of PEM (Nafion 117) was 12 cm^2. The cathode was made from woven graphite fibers (University of Reading, Department of Microbiology, UK) and the cathodic chamber was filled with 0.5 M potassium thiocyanate and 0.5 M phosphate buffer. The working temperature was 20 °C. No chemical catalysts were used in either chambers of the microbial fuel cell. The MFC were fed with modified LB medium (1 g/L glucose, 5 g/L yeast extract, 10 g/L NaCl and 1 g/L tryptone).

In semi-continuous operation, the medium volume was